MAX-PLANCK-GESELLSCHAFT

Future Plan[t]s

7th - 9th September 2015

Max Planck Institute
of Molecular Plant Physiology
Potsdam, Germany

Topics and Discussion

Plant Evolution and Development

Metabolism and Signalling

Biotechnology and Engineering

The future of science and of the scientist:
Career paths in academia and beyond

Organised by PhD students

IMPRS
Primary Metabolism and Plant Growth

Universität Potsdam

Max-Planck-Institut für Molekulare Pflanzenphysiologie

Plants and People

Future Plan[t]s

Welcome to Plants and People 2015!

Plants and People Conferences are organised by the PhD students at the Max Planck Institute of Molecular Plant Physiology in Potsdam-Golm, and occur every second year.

Our conferences aim to bring together a unique mixture of high-profile international speakers, to not only present on their specific research within the plant science field, but to also discuss wider aspects of life and growth within the scientific research world.

This year's Plants and People theme is **Future Plan[t]s.** We hope that this topic will allow us to focus not only on recent developments in plant science, but also to delve into the aspects involved in the development and growth of the plant *scientist* themselves, and his or her career – an issue close to the hearts of the organising Phd students.

Thank you!

Plants and People 2015 is financially supported by the International Max Planck Research School 'Primary Metabolism and Plant Growth' (IMPRS-PMPG). IMPRS-PMPG is a joint doctoral programme of the University of Potsdam and the Max Planck Institute of Molecular Plant Physiology.

Thank you to all the helpers on and leading up to the conference days for their support, Dr. Ina Talke and Dr. Ulrike Glaubitz and the administration of the MPI-MP for support in planning and organising, Stefan Heinrich and Jan Scharein for web design, and the 2011 P&P team for logo design, and for breaking the ground.

Many thanks to Roboklon, NEB, Sigma-Aldrich and Macrogen for sponsoring our meeting.

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Max Planck Institute of Molecular Plant Physiology
Science Park Potsdam-Golm
Am Mühlenberg 1 • D-14476 Potsdam • Germany

http://plants-and-people.mpg.de/

Conference Programme

Time	**Monday, 07.09.**
12:30	**Registration**
14:00 – 16:00	**Regulation**
14:00	Fabio Fornara
14:40	Claudia Köhler
15:20	Iris Finkemeier
16:00	*Coffee break*
16:20 – 17:40	**Plant Evo/Devo**
16:20	Claudio Varotto
17:00	Stephen I. Wright
17:40	*Break*
18:00 – 19:00	**Keynote Lecture**
	Thomas Börner
19:00	**Poster Session** (with light refreshments)

Time	**Tuesday, 08.09.**
09:30 – 11:50	**Metabolism and Signalling**
09:30	Ilka Axmann
10:10	Ute Roessner
10:50	*Coffee break*
11:10	Elizabete Carmo Silva
11:50	*Lunch (not provided)*
13:00 – 14:30	**Alumni in Companies**
13:00	Dörthe Dräger
13:30	Henning Redestig
14:00	Martina Schad
14:30 – 15:10	**Metabolism and Signalling** Cont.
14:30	John Bothwell
15:10 – 16:15	**Poster Session** (with coffee break)
16:15 – 17:45	**Discussion Session**
	The future of science and the future of me
	Moderated by Claudia Steinert
18:00	**BBQ**

Time	**Wednesday, 09.09.**
09:30 – 12:30	**Biotechnology and Engineering**
09:30	Mansour Karimi
10:10	Nethaji Gallage
10:50	*Coffee break*
11:10	Stefan Schillberg
11:50	Ralf Reski
12:30	**Concluding remarks and Poster Prize**
13:00	*Lunch (not provided)*

Conference Venue

Max Planck Institute of Molecular Plant Physiology

Our institute was established in 1994, with founding director Prof. Dr. Lothar Willmitzer focusing investigations on plant central metabolic pathways and analysis of plant gene function. In the 21 years that have followed, we have grown to include directors Prof. Dr. Mark Stitt and Prof. Dr. Ralph Bock, independent and associated group leaders, and over 300 postdocs, students, technicians and support staff originating from all corners of the world.

The research focus, too, has shifted with the times. Our long-term goal is to develop a comprehensive, systems-level understanding of the plant: to not only understand the underlying genetic factors that drive plant growth, but to also asses the dynamics of plant responses. To achieve this, we combine analyses at multiple levels – integrating fuctional analysis of individual genes and specific molecular details, with generated 'omics' data sets, network models, and existing biological knowlege.

For more details about the Max Planck Institute of Molecular Plant Physiology, our people and our science, please visit our website: www.mpimp-golm.mpg.de

Travel Information

Deutsche Bahn has announced changes in the regional train schedules for the conference dates. Please visit http://bauarbeiten.bahn.de/berlin-bb for further information.

Connections between Golm and Potsdam Hauptbahnhof:

Bus:	**605** Wissenschaftspark Golm <> S Hauptbahnhof Potsdam
	606 Alt-Golm <> S Hauptbahnhof Potsdam
Train:	**RB 21** Bahnhof Golm <> S Hauptbahnhof Potsdam
	RB 22 Bahnhof Golm <> S Hauptbahnhof Potsdam

Trains from Berlin central train stations (Friedrichstrasse, Hauptbahnhof, Zoologischer Garten, Charlottenburg):

RB 21 and **RB 22** (to Wustermark or Berlin-Schönefeld, respectively): Direct trains from Berlin to Golm every half hour, approximately between 7:00 and 9:00 in the morning and 15:00 and 18:00 in the afternoon.
At other times during the day, **RE 1** (to Brandenburg) goes to Potsdam-Hauptbahnhof every 30 minutes. In Potsdam Hbf., you change to the RB 21 or RB 22. The **S 7** goes between Potsdam Hbf. and Berlin stations.

It is a 10 min walk between the Golm train station and the Max Planck campus. For further information, please visit our website.

Max Planck Campus Map

All lectures take place in the lecture hall of the Max Planck campus, located in building 4 (central building: yellow). The poster session will take place in the foyer of the central building.

Canteen/cafeteria: Max Planck canteen in building 4
Fraunhofer canteen near building 7

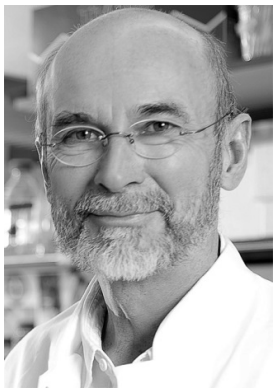
At the Max Planck canteen you pay with a pre-paid card that you can get from the machines at the entrance of the canteen. At the Fraunhofer canteen you can pay in cash.

Speaker

Profiles & Abstracts

Keynote Lecture

Thomas Börner



Institute of Biology, Department of Genetics, Humboldt University Berlin, Germany

Thomas Börner is a plant geneticist who has had a crucial impact on the plant science field. He began his outstanding scientific career at the Martin Luther University in Halle-Wittenberg, completing his PhD in the lab of Rudolf Hagemann in 1974. He continued with his habilitation in genetics, which he completed in 1979, and then moved to Berlin, where he took a position as an Associate Professor at the Humboldt University. From 1982 he was the head of Genetics at the HU, and two years later became a professor for genetics, working from 1993 until 2012 as a C4 professor. His research interests are widespread, with a focus on the genomes of chloroplasts and mitochondria, particularly their gene expression and interactions with the nucleus. Thomas Börner has engaged himself in numerous scientific activities, for example the German Academy of Sciences Leopoldina and the Collaborative Research Center 429, for which he is Spokesman.

Abstract

Genes and career. Or: How to become a prof.

I will describe how I found my ways from chloroplasts to mitochondria and cyanobacteria and back to chloroplasts and from Halle to Berlin. And I will try to identify ‘endogenous and exogenous factors’ that have guided me on these ways.
Finally, I will explain why I am optimistic about the future of plant science but at the same time pessimistic about my abilities to predict how this future will look.

Fabio Fornara

Department of Biosciences, University of Milan, Italy

Fabio Fornara received his PhD in Plant Biology from the University of Milan in 2005 and shortly after moved to the Max Planck Institute for Plant Breeding Research in Cologne (MPIPZ) to join the group of George Coupland as a postdoctoral fellow. At the MPIPZ he became interested in the mechanisms that plants use to sense day length and promote reproductive development. In 2011, taking advantage of a Starting Grant from the European Research Council, he returned to Italy and started his own lab at the Department of Biosciences of the University of Milan. His scientific interests include the mechanisms of diurnal and seasonal time measurement in rice, the response of the shoot apical meristem to floral inductive cues, and the molecular processes leading to artificial adaptation of a tropical species to high latitudes. In 2014, he became Associate Professor at the University of Milan where he is currently teaching Plant Biology and Plant Developmental Biology.

Abstract

Heading north! Regulatory networks controlling photoperiodic flowering in rice

Flowering of rice (*Oryza sativa*) is promoted when plants are exposed to short day lengths (SDs) and repressed upon exposure to long ones (LDs). Changes in day length are perceived by regulatory molecules in the leaves that under SDs promote the production of long-distance systemic signals called florigens. After domestication, expansion of rice cultivation to higher latitudes has been accompanied by artificial selection of varieties with reduced sensitivity to day length and increased expression of the florigens, to allow flowering under non-inductive conditions. Taking advantage of varieties cultivated in Mediterranean Europe, we are exploring the genetic variation contributing to photoperiod insensitivity and necessary to adapt rice to environments different from those of initial domestication. The analysis of novel alleles of *HEADING DATE 1* (*Hd1*), a central regulator of rice flowering, indicates how artificial selection has operated at different levels of gene expression to impinge on photoperiod sensitivity.

The rice genome encodes at least two florigens, *HEADING DATE 3a* (*Hd3a*) and *RICE FLOWERING LOCUS T 1* (*RFT1*), whose cognate proteins move to the shoot apical meristem (SAM) and induce extensive changes in gene expression. During this process, the SAM modifies its developmental program and forms an inflorescence called panicle. We have quantified the dynamics of global gene expression in SAMs of two rice cultivars showing distinct responses to photoperiodic induction, and identified several regulatory genes whose expression is dependent upon florigenic proteins. *DOWNREGULATED DURING TRANSITION 1* (*DDT1*), a transcription factor belonging to the C2H2 zinc finger family, is strongly repressed at the meristem as soon as plants are exposed to inductive day lengths. Transgenic rice plants in which *DDT1* expression is artificially modified by distinct promoters, or gene function is abrogated through targeted editing of the locus, indicate a role for *DDT1* in flower differentiation and internodes elongation. These data suggest the existence of a floral repressor program at the SAM that limits the vegetative-to-reproductive phase change.

Claudia Köhler

Uppsala Biocenter, Swedish University of Agricultural Sciences and Linnean Center of Plant Biology in Uppsala, Sweden

Claudia Köhler received her PhD in 1999 from the University of Freiburg, Germany, followed by postdoctoral training in Ueli Grossniklaus' lab at the University of Zurich, Switzerland. In 2005, she was appointed as assistant professor at the ETH Zurich and accepted an offer as full professor from the Swedish Agricultural University in Uppsala in 2010. The main research interest of Claudia Köhler is in genetic and epigenetic mechanisms governing seed development and their impact on plant speciation. Her research revealed that the endosperm has a major role in establishing postzygotic hybridization barriers between plants of different ploidy as well as between related plant species. This barrier is built by deregulated expression of imprinted genes that are expressed dependent on their parent-of-origin. Imprinted genes receive their "imprint" during gametogenesis, where specific epigenetic modifications are applied that are maintained during endosperm development. The current interest is to identify those modifications and to understand the mode of their establishment.

Abstract

Epigenetic mechanisms in the endosperm drive plant specication

Polyploidization is a widespread phenomenon among plants and is considered a major speciation mechanism. Polyploid plants have a high degree of immediate post-zygotic reproductive isolation from their progenitors, as backcrossing to either parent will produce mainly nonviable progeny. This reproductive barrier is called triploid block and it is caused by malfunction of the endosperm. Our work has revealed that deregulated parent-of-origin specific genes (imprinted genes) are causal for the response to interploidy hybridizations, revealing an epigenetic basis of this phenomenon. I will discuss epigenetic changes in response to interploidy hybridizations and their consequences for endosperm development. I will furthermore discuss an epigenetic method for the generation of viable triploids, providing an impressive example for the potential of epigenome manipulations for plant breeding. Lastly, I will discuss a recently evolved interspecies hybridization barrier in the genus Capsella that reveals striking similarities to interploidy hybridization barriers, suggesting a common mechanistic basis.

Iris Finkemeier

Institute for Plant Biology and Biotechnology, WWU Münster and
Max Planck Institute for Plant Breeding Research, Cologne, Germany

Iris Finkemeier received her PhD in Plant Biochemistry from the University of Bielefeld in 2005, where she worked in the group of Prof. Karl-Josef Dietz. Shortly afterwards she was awarded a Feodor-Lynen Research Fellowship from the Alexander von Humboldt Foundation to work at the Department of Plant Sciences with Dr. Lee Sweetlove and Prof. Christopher Leaver, University of Oxford (UK). In 2007, she was awarded a Junior Research Fellowship from Christ Church College, which allowed her to continue the research in Oxford. In 2010, she moved from Oxford to Munich to start a young investigator group at the LMU funded by the Emmy Noether program of the DFG. Her group was associated to the department of Prof. Dario Leister where she performed her habilitation in Botany. In 2014, she moved with her Emmy Noether group to the MPIPZ as head of the Plant Proteomics and Mass Spectrometry facility. In 2015, she was appointed as W2 Professor for Plant Physiology at the WWU Münster. Her goal is to identify novel regulators of plant metabolism and energy signaling pathways.

Abstract

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

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 (Choudhary, 2009). Until recently, almost nothing was known about this modification in plants beyond the well-studied acetylation of histone proteins in the nucleus (Finkemeier *et al.*, 2011; Wu *et al.*, 2011). We developed a protocol based on high resolution mass spectrometry for the identification and relative quantification of lysine-acetylated peptides (K-ac) in *Arabidopsis thaliana*. 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.

Claudio Varotto

Biodiversity and Molecular Ecology Department, Fondazione Edmund Mach, San Michele all'Adige (TN), Italy

Claudio Varotto carried out his PhD in photosynthesis functional genomics at the Max Planck Institute for Plant Breeding Research in Cologne (MPIPZ, Germany) in the Group of Dario Leister. After receiving his PhD in Botany from the University of Cologne in 2002, he carried out his first postdoc in the group of Heinz Saedler, where he developed his interest for plant evolution and biodiversity. In 2004 he became project leader at the Fondazione Edmund Mach of San Michele all'Adige (Trento, Italy), where he was hired as staff researcher and started his own group. In the following years he established comparative genomics approaches in a phylogenetic framework for the study of plant adapative processes in wild and domesticated species from the model plant families Brassicaceae and Ranunculaceae. More recently, he applied the same approaches also to wild and semi-domesticated species from the Arundineae tribe of the Poacaea family, with a special focus on *Arundo donax*, a promising biomass and bioenergy species.

Abstract

Genomic signatures of specialized metabolism in plants

Arundo donax, also known as the giant reed, is recognized as one of the most promising non-food bioenergy crops in southern Europe. Despite its relevance, until recently no genomic resources were available to support the characterization of the developmental, adaptive and metabolic traits underlying the high productivity of this non-model species. With the goal of partly filling this gap of knowledge we recently carried the de novo assembly of bud, culm, leaf and root transcriptomes of *A. donax* for mining and exploring the genetic potential of this species.

The analysis of the transcriptome reveals strong differences in the enrichment of the gene ontology categories and the relative expression among different organs, which can guide future efforts for metabolic engineering of *A. donax*. A set of homologs to key genes involved in lignin, cellulose, starch, lipid metabolism and in the domestication of other crops is discussed to provide a platform for possible enhancement of productivity and saccharification efficiency in *A. donax.* We further obtained by RNA-seq the whole root and leafshoot transcriptomes of young *A. donax* plants subjected to two diffreent intensities of water stress, and identified a total of 3034 differentially expressed genes. Mining of stress-related genes indicated the higher responsivity of roots compared to shoots at the early stages of water stress especially under the milder water stress, with a majority of genes responsive to salt, oxidative and dehydration stress. Analysis of gene ontology terms underlined the qualitatively different responses between root and shoot tissues. In root DEGs were associated mainly to polysaccharide catabolism and biotic stress, in line with responses to osmotic stress and incipient cellular damage. In shoot early water stress was instead dominated by functions related to signal transduction and protein modification associated to phosphorylation, indicating a milder/delayed onset of stress. Analysis of metabolic pathways highlighted the crucial role played in both shoots and roots by genes involved in the signalling cascade of abscissic acid. We further identified relatively large organ-specific differences in the patterns of drought-related transcription factor AP2-EREBP, AUX/IAA, MYB, bZIP, C2H2 and GRAS families, which may underly the transcriptional reprogramming differences between organs. Through comparative analyses with major Poaceae species, we finally identified a set of 53 orthologs that can be considered as a core of evolutionary conserved genes important to mediate water stress responses in the family.

Stephen I. Wright

Department of Ecology and Evolutionary Biology, University of Toronto, Canada

Stephen Wright is an Associate Professor in the Department of Ecology and Evolutionary Biology at the University of Toronto. His research focuses on plant population genomics, with a particular emphasis on characterizing the extent and nature of genome-wide natural selection across the genome. A major direction in his lab has been on studying the causes and genomic consequences of mating system evolution.

Abstract

When history repeats itself: Population genomics of degenerative genome evolution

One of the most striking features of genome evolution concerns cases where large-scale genomic regions experience widespread gene degeneration and loss. This includes sex chromosome degeneration and diploidization following whole genome duplication, where gene copies that once shared common ancestry undergo degenerative evolution. Such patterns are surprising, since they reflect a dramatic loss of selective constraint.

Although the features of degenerative genome evolution have been well documented, much of the focus has been on ancient events, making it difficult to investigate the processes driving the earliest stages of genome degeneration. Plants often experience recurrent transitions in ploidy and mating system, allowing for important opportunities to investigate these early stages of genome evolution. Here, I discuss our work investigating how rapidly plant populations experience major shifts in selective pressure following transitions in mating system and ploidy. The results suggest the potential for rapid and major shifts in selection pressure following evolutionary transitions to self-fertilization, polyploidy, asexual reproduction and sex chromosome formation. Large-scale comparative plant genomics efforts will enable powerful replicated tests of the factors governing differences in selection across lineages.

Ilka Axmann

Heinrich Heine University Düsseldorf, Institute for Synthetic Microbiology, Düsseldorf, Germany

Prof Axmann has been working interdisciplinary on molecular-genetic approaches, bioinformatics and systems biology of bacterial regulatory networks for more than 15 years. In 2013, she was appointed a Junior Professorship for Synthetic Microbiology at the Heinrich-Heine University Duesseldorf, within the Cluster of Excellence on Plant Sciences (CEPLAS). Her current research is aiming on smart, automated and dynamic control of synthetic metabolic pathways in microorganisms using de-novo designed RNA-devices and RNA-based metabolite-sensors. Particular focus is placed on the engineering of cyanobacteria as a future host for sustainable biotechnology.

Abstract

Cyanobacteria's specific features: non-standard circadian clocks and plenty of antisense RNAs

Cyanobacteria possess a circadian clock system that consists of mainly three proteins: KaiA, KaiB and KaiC control daily cycles of gene expression and chromosome compaction. Our previous analyses revealed that complex formation between Kai proteins and, therefore, their stoichiometry is essential in maintaining robust circadian oscillations. Thus, it is puzzling that the chromosomes of many cyanobacteria contain multiple kai-gene copies including the cyanobacterium *Synechocystis sp.* PCC 6803.

Interestingly, our global transcriptomic analyses of light-dark synchronised *Synechocystis* cultures indicate a rather light-driven than a circadian regulated pattern in global gene expression. We detected several small RNAs encoded at the kai gene loci but antisense to kai genes which might be involved or even interfere with circadian regulation. Besides several other studies, we have already shown how small RNAs can influence the temporal regulation of gene expression. Thus, regulation by antisense RNA might be a fundamental mechanism for the temporal coordination of gene expression in cyanobacteria.

Ute Roessner

School of Botany, University of Melbourne, Australia

Prof. Ute Roessner obtained her PhD in Plant Biochemistry at the Max Planck Institute of Molecular Plant Physiology in Germany, where she developed novel GC-MS based methods to analyse metabolites in plants. With the combination of small molecule analytics and sophisticated bioinformatics and statistics, the field of metabolomics was born, which today is an important tool in biological sciences, systems biology and biomarker discovery. In 2003, she moved to Australia where she established a GC-MS and LC-MS based metabolomics platform as part of the Australian Centre for Plant Functional Genomics (www.acpfg.com.au). Between 2011 and 2014 she led the ACPFG node at the School of BioSciences, The University of Melbourne. Also in 2007, Ute became involved in the setup of Metabolomics Australia (MA, www.metabolomics.com.au), a member of BioPlatforms Australia (www.bioplatforms.com.au), and now leads the MA node at the School of BioSciences, The University of Melbourne. In 2013, she was awarded a prestigious ARC Future Fellowship which aims to identify novel mechanisms of salinity tolerance in barley by spatial analysis of metabolites and lipids using Imaging Mass Spectrometry.

Abstract

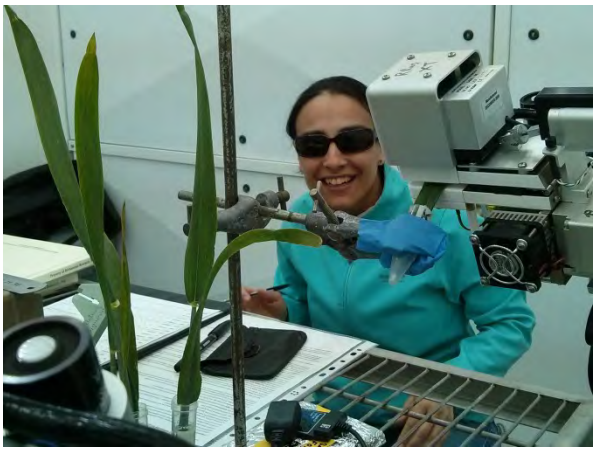
Spatially resolved systems biology to identify novel salinity tolerance mechanisms in barley roots

Barley (*Hordeum vulgare L.*) is an essential food and brewing crop. As a glycophyte, it suffers substantial yield loss when grown under saline conditions. Relatively little is currently understood of salt stress perception and responses in plant roots, which involve complex changes at the physiological, metabolic, molecular, transcriptional, and genetic level.

We aim to develop new tools to unravel how plants respond to the perception of salt stress. Evidence is accumulating that lipid signalling is an integral part of the complex regulatory networks in the responses of plants to salinity through modifications of membrane lipids, which occur through the activity of phospholipases, lipid kinases and phosphatases such as phospholipase D and diacylglycerol kinase that produce different classes of lipid and lipid-derived messengers. These provide spatial and temporal regulatory functions crucial for cell survival, growth and for an appropriate response of the plant to environmental stimuli. Initial analyses indicate that different tissue types within the root respond differently to salt stress in tolerant and sensitive cultivars. Here, we study the root responses to salinity using a combination of next generation RNA-sequencing, cell wall composition analysis and targeted metabolite and lipid analyses of three key sections of barley roots (root cap and cell division zone, elongation zone, and maturation zone). In addition, we are using modern lipidomics technologies to compare the root plasma membrane (PM) compositions of different barley genotypes with contrasting salinity tolerance levels upon salt stress. We also use MALDI-FT-MS based imaging technologies to monitor spatial distributions of metabolites and lipids across root sections of salt-treated tolerant and sensitive barley genotypes. Transcriptomics results are now being integrated with spatial biochemical data, enhancing our understanding of system-wide and tissue-specific responses of roots to salinity stress.

Given the lack of fundamental knowledge of the genes and proteins involved in signalling, cell wall and lipid metabolism under salinity stress, and the enormous potential for biotechnological application in this area, our results provide insight into novel mechanisms responsible for salt tolerance of barley.

Elizabete Carmo-Silva



Rothamsted Research, UK
From September 2015: Lancaster Environment Centre, Lancaster University, United Kingdom

Elizabete Carmo-Silva was born and grew up in Sintra, Portugal. She studied Applied Plant Biology at the University of Lisbon before starting a PhD in Plant Physiology and Biochemistry, awarded by the same University and in collaboration with Rothamsted Research, in the UK. She then did a four-year postdoc with the United States Department of Agriculture in Arizona and, in 2012, moved back to Europe and Rothamsted Research with a tenure-track research scientist position. In September 2015, she will take up a lecturer position at the University of Lancaster, in the UK. Her research focuses on photosynthesis, with an emphasis on the regulation of CO_2 fixation by Rubisco. She aims to contribute to improving food security by optimising crop performance in response to the changing climate.

Abstract

Improving the efficiency of Rubisco and CO_2 assimilation

Crop yields must increase at faster rates than those currently observed in breeding programmes. Moreover, this needs to happen without increasing land use or negative impacts on the environment, to ensure future global food security in a sustainable way. Photosynthetic CO_2 assimilation is the main determinant of plant biomass production, but it is a relatively inefficient biological process with scope for substantial improvement. We are exploiting existing genotypic variation in photosynthesis and crop yields to inform breeding of higher yielding wheat cultivars and, in parallel, we are using genetic engineering as a tool to optimize photosynthetic productivity in crop plants.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) plays a key role in CO_2 assimilation, but the enzyme is not optimally poised for crop productivity in current and projected climates. We are characterising the catalytic properties of Rubisco from diverse genotypes to identify enzymes with superior performance for improving CO_2 assimilation in specific crops and environments. Rubisco is prone to inhibition by the unproductive binding of sugar-phosphates that lock active sites in a closed conformation. Re-activation of Rubisco in vivo depends on the interaction with its catalytic chaperone, Rubisco activase (Rca). Rca uses the energy from ATP hydrolysis to facilitate the release of sugar phosphate inhibitors from Rubisco catalytic sites. The regulation of Rubisco activity, via interaction with Rca, affects the response of CO_2 assimilation to environmental conditions experienced by plants in the field. Recent evidence shows that a potential increase in biomass production under fluctuating light can be obtained by modifying Rubisco regulation by Rca in the model plant species Arabidopsis, suggesting that this can be a promising strategy for crop yield improvement.

Our research aims to understand and characterise the functional significance of existing variation in the properties of Rca isoforms, and to improve the light and temperature response of Rubisco activation to optimise CO_2 assimilation and crop yields.

Dörthe Dräger

Rijk Zwaan, Wageningen, Netherlands

After her studies of agricultural biology at the Hohenheim University, she started her PhD at the MPI in Golm in the research group of Dr. Ute Krämer in December 2004. In her project she studied the molecular and genetic mechanisms of heavy metal tolerance in a wild Arabidopsis species. Following up on her PhD, she spent 3 years (2005-2007) as a postdoctoral researcher at the University of Georgia, USA, in the lab of Dr. Steven Knapp, focusing on molecular breeding in sunflower. At the beginning of 2008, she returned to Europe to work as the tomato pre-breeder at Rijk Zwaan. In May 2014, she had the possibility to change departments within the company and is now working as a researcher in the department of Quantitative Genetics.

Abstract

Rijk Zwaan

Rijk Zwaan is an independent, international organization specialized in the breeding, production and selling of vegetable seeds. Rijk Zwaan is a totally independent family business with employee participation. 86% of the shares are in the hands of three families and 14% of the capital is in the hands of employees in the form of share certificates. Our head office is located in De Lier, The Netherlands. In addition, we have 30 subsidiaries in 26 different countries, where we carry out our sales, R&D and seed production. Currently, Rijk Zwaan counts 2,350 employees who work in many different locations all over the world. Over 1,000 of them work in the Netherlands. Our seeds are sold in more than 100 different countries all over the world, via 27 locally-operating Rijk Zwaan sales subsidiaries and numerous (exclusive) distributors. We have more than 1,000 varieties in our assortment in 25 different vegetable crops. Among these are fruit vegetables like tomato, pepper, cucumber and eggplant as well as outdoor crops like lettuce, spinach, cabbage, carrots, …

One of Rijk Zwaan's main activities focuses on the creation of new vegetable varieties. The wishes and demands made worldwide by growers, the vegetable trade, processing industry and consumers form the starting point at Rijk Zwaan for our breeding activities. Some examples of these are high yield and long shelf life after harvesting, but also germination vigour of the seed and resistance to various diseases. Consumers are especially interested in an attractive and tasty product and like to be surprised with new colours and shapes.

The breeding activities are supported by new technologies, which have been implemented in the breeding process. Research activities take place in the fields of molecular biology, phytopathology, seed technology, cell biology, and biochemistry. Together with a number of other vegetable seed companies, Rijk Zwaan is shareholder of Keygene N.V., a research company located in Wageningen, NL, where research is collectively carried out in the area of modern biotechnology. We also collaborate frequently with universities and research institutes.

Henning Redestig

Bayer CropScience, Ghent, Belgium

Henning Redestig did his PhD at the Max Planck Institute of Molecular Plant Physiology, in Golm, Germany. He then went to Japan for a postdoc at the RIKEN Plant Science Center. He joined Bayer CropScience in 2011.

Abstract

Navigating between industry and academia, model and crop genomes

After finishing my PhD on integrative approaches for gene expression data analysis at the MPI-MP, and a postdoc in the RIKEN Plant Science Center, I joined Bayer CropScience in 2011. Here I am a senior scientist performing data analysis, algorithm and tool development to support trait research and discovery. At BCS, we are improving agronomically important traits to help meeting the ever growing demands of increased crop production. Trait discovery is the search for new leads (genes, phenotypic traits, molecules) that have the potential to be beneficial for agriculture. In this process, we combine data from diverse species in an integrative fashion.

In my talk, I provide an account of my transition from academia to industry, how we collaborate with academia to develop open computational biology solutions and how these support our trait discovery activities.

Martina Schad

OakLabs, Henningsdorf, Germany

Martina Schad obtained her PhD in Biochemistry at the Max Planck Institute of Molecular Plant Physiology in Golm, Germany, in 2005. In her thesis she analysed tissue-specific samples comprehensively on three "omics" topics. As her first career step, she became product manager of NimbleGen microarray services at RZPD (German Resource Centre for Genomics). In 2007, Martina Schad became head of division at the newly formed imaGenes, a spin-off of RZPD. In this role, she successfully established several new services offers in the domain of RNA and DNA analysis. In 2011, Martina Schad founded her own company together with the quantum physicist Dr. Jim Kallarackal: OakLabs offers products and services for the life science sector by establishing an interface between genomics and algorithms. Today, the company has 10 employees and works with customers worldwide.

Abstract

Successful in Life Sciences: Why building your own company should always be an alternative

The startup scene has seen a number of interesting new companies and new ideas in the last couple of years. Still only an ever so small minority of graduates even considers starting a company from scratch.

Alternative: Startup

While it is certainly valuable to get insights into a company as an employee, many do not even think about the alternative, may it be fear of the unknown, insecurity or the financial situation.

Seen from the viewpoint of a successful start-up founder with a successful life sciences company that has been growing ever since its formation in 2011, a startup should always be considered as an alternative, it offers many chances which have to be weighed against the risks. Previous experience from another company only helped to make full use of the potential from the start.

Benefits:

- Valuable insights about company structures
- Possibility to discover strengths one never knew of
- Possibility to get to know lots of different fields far from the own studies: marketing, sales, HR, finance etc.
- Chance to develop a business environment according to one's own liking and to work with the people one would like to work with
- Contact to and new input from investors, business partners, other startups around the world

Conclusion:

While it is not necessary that everyone starts a company directly after graduating or ever at all, it is important to see this as a possible step and real alternative in the long run. The result can be new learnings, great chances and a hopefully successful career.

John Bothwell

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John Bothwell is a Reader (Assoc. Prof.) in Bioenergy at the University of Durham, where he works on the evolution, systematics, and biotechnological applications of seaweeds. John's DPhil in Biochemistry was awarded from Oxford University in 2000 and between 2000-2007 he held postdoctoral research fellowships in Cambridge and the Marine Biological Association (Plymouth), taking a year off in 2002-2003 to play professional rugby. From 2009-2012, he held a Lectureship at Queen's University Belfast and moved to Durham in 2013.

Abstract

Red, Green, Brown, and Blue: What are the prospects for seaweed biotechnology?

The macroalgae, or seaweeds, are an attractive biomass source for biotechnology and bioenergy applications. They grow fast, do not compete for land with food crops, and can bioremediate marine waste streams. However, because green, red, and brown seaweeds are evolutionarily distinct from land plants, they have characteristic biomass compostions (high in protein and ash, with algal-specific structural and storage carbohydrate polymers) that make them less amenable to processing by existing biotechnology pathways.

Our work looks at integrated approaches to growing and processing seaweed biomass, using the green *Ulva* spp. as a model (the sea lettuces). Our approach involves: a) GWAS (Genome-Wide Association Studies) of *Ulva* strains using RADseq to find markers associated with desirable traits, b) selective breeding of *Ulva* strains using marker assisted selection, c) sequential extraction and biorefining of higher-value components of green seaweed biomass (e.g. ulvan, minerals), d) fermentation screens of culturable bacteria to identify marine bacterial strains capable of degrading and processing the individual components of green seaweed biomass.

Seaweed biotechnology is in its very early stages, but work of this sort is now beginning to provide the evidence needed to consider how coastal ecosystems can support sustainable biomass production.

Mansour Karimi

VIB, University of Ghent, Belgium

Mansour Karimi, a senior scientist at VIB (Ghent, Belgium), did his PhD in plant biotechnology at Ghent University, Belgium. In his PhD he worked in promoter tagging to identify nematode inducible plant promoters. Since his PhD he has been involved in several research projects related to plant Biotechnology. In 2002, he started to develop Gateway-compatible plant binary vectors. Due to his interest in the Gateway cloning system, he developed this system for monocots and *Physcomitrella patens*. Besides developing vectors, he was involved in the Arabidopsis silencing project (AGRIKOLA) and currently is working on the genome editing technology CRISPR/Cas system.

Abstract

Gateway-compatible vectors for plant functional genomics

The Gateway cloning system exploits the site-specific recombination reactions used by bacteriophage λ to shuttle itself in a host bacterial chromosome. We have constructed a large collection of Gateway-compatible destination vectors for a wide range of gene function analyses in transgenic plant cells. Using MultiSite recombination Gateway cassettes, plant binary destination vectors have also been created in which two or three segments can be transferred contiguously or in independent expression unit, in a single LR clonase in vitro reaction. Our binary destination vectors carry one of three plant selectable markers coding for resistance to kanamycin (nptII), hygromycin (hpt) or glufosinate ammonium (bar). Destination vectors are available in *E. coli* high copy number plasmids.

To further streamline the construction of recombinant genes, we have built series of reference Gateway entry clones carrying promoters, terminators, and reporter open reading frames most commonly used in plant research. They are interchangeable, fully documented, and can be combined at will according to the desired output.

We added to our resources a novel series of Gateway binary vectors that facilitate the modular assembly of genes of interest together with new regulatory sequences, such as strong constitutive or endosperm-specific *Brachypodium distachyon* promoters. This resource aims for creation of vectors and transgenes designed to explore gene functions in monocotyledonous crops. Recently we have developed a Golden Gateway cloning system, this system facilitates assembly of a multi-component DNA constructs and can be used for gene stacking and gene combination approaches.

The Gateway entry clones and destination vectors can be obtained on line (http://www.psb.ugent.be/gateway). This web site provides recombinational cloning instructions, as well as experimentally verified sequences, maps and Vector NTI files for each plasmid.

Karimi *et al.* (2002) GATEWAY vectors for Agrobacterium-mediated plant transformation. Trends Plant Sci. 7:193-195.

Karimi *et al.* (2005) Modular cloning in plant cells. Trends Plant Sci. 10:103-105.

Karimi *et al.* (2007) Building blocks for plant gene assembly. Plant Physiol. 145:1183-1191.

Coussens *et al.* (2012) *Brachypodium distachyon* promoters as efficient building blocks for transgenic research in maize. J. Exp. Bot. 63, 4263-73.

Karimi *et al.* (2013) Gateway vectors for transformation of cereals. Trends Plant Sci. 18: 1-4.

Nethaji Gallage

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Nethaji Gallage was born in Sri Lanka. She completed her Bsc and Msc studies at the University of Copenhagen, before starting a PhD at the Plant Biochemistry laboratory, under supervision of Prof. Birger Lindberg Møller. During her PhD she characterized one of the main enzymes involved in the vanillin biosynthesis in vanilla, work which led to multiple publications and a patent.

Abstract

Pathway discovery of the most popular plant flavour compound vanillin

Vanilla and its key flavour component vanillin, a universally appreciated flavour, global delicacy and probably the most popular plant natural product, is derived from the seedpods of the orchid *Vanilla planifolia* and other related species. The flavour and fragrance profile of the vanilla extract contains more than 200 components. Vanillin (3-methoxy-4-hydroxybenzaldehyde) is the main flavor compound in the vanilla extract and is the basis and an additive of sweets, ice creams, soft drinks and many more products in the food, beverage and pharmaceutical industry. Despite its popularity, the biosynthetic pathway of vanillin has remained elusive until now.

A single hydratase/lyase type enzyme designated vanillin synthase (VpVAN) catalyzes direct conversion of ferulic acid and its glucoside into vanillin and its glucoside, respectively. The enzyme shows high sequence similarity to cysteine proteinases and is strictly specific to the substitution pattern at the aromatic ring. Transient expression of VpVAN in tobacco and stable expression in barley in combination with the action of endogenous alcohol dehydrogenases and UGTs result in vanillyl alcohol glucoside formation from endogenous ferulic acid. A gene encoding an enzyme showing 71% sequence identity to VpVAN was identified in another vanillin producing plant species Glechoma hederacea and was also shown to be a vanillin synthase as demonstrated by transient expression in tobacco[1]. Pathway discovery of vanillin biosynthesis has constructed the base for establishing a vanillin biosynthetic pathway in yeast exclusively based on Vanilla orchid genes which is our main research focus at the moment.

In 2010, the annual global sales of vanillin reached more than 15,000 tons. Nowadays, it is less than 1% of the global production of vanillin is derived from vanilla pods while the majority is produced synthetically using e.g. lignin and eugenol as starting materials. The production of vanilla pods and the isolation of vanillin from vanilla pods is a laborious and costly process. Production of 1 kilogram (kg) of vanillin requires approximately 500 kg of vanilla pods corresponding to the pollination of approximately 40,000 vanilla orchid flowers. This is why; a huge surge in the exploration of more environmentally friendly biosynthetic procedures to make natural flavours has risen. Moreover, Industrial application of bioengineered microorganisms for vanillin production has gained quite a lot of attention not only from the flavour and fragrance industries, but also from environmental groups, the general public and politicians. ([2], http://theplate.nationalgeographic.com/2014/10/23/plain-vanilla/)).

The recent identification of VpVAN from the vanilla orchid can contribute to an entirely new opportunity for biotechnology based production of natural vanillin and as well as vanillin based compounds such as capsaicin and capsaicinoids (pungency compounds in chili and have pharmaceutical importance). If high expression levels can be obtained in yeast production strains and the enzyme is stable and has proper kinetic characteristics, this may constitute an alternative to the current yeast production systems.

[1]Gallage, N.J., Hansen, E.H., Kannangara, R., Olsen, C. E., Motawia, M. S., Jørgensen, K., Holme, I., Hebelstrup, K., Grisoni, M., Møller, B.L. Vanillin formation from ferulic acid in Vanilla planifolia is catalysed by a single enzyme. Nature Communications 5, doi:10.1038/ncomms5037 (2014). I.F. 10,742

[2]Gallage, N.J. and Møller, B.L. Vanillin – Bioconversion and Bioengineering of the most popular plant flavour and its de novo biosynthesis in the vanilla orchid. Molecular Plant, special edition Synthetic Biology doi: 10.1093/mp/ssu105 (2014).

Nethaji J. Gallage, Esben Halkjaer Hansen, Birger Lindberg Møller and Jørgen Hansen. Microbial organism and methods for producing vanillin, vanillyl alcohol, or vanillin glucoside, by vanillin synthase action on ferulic acid. International Patent Application PCT/DK2013/050357-27.

Stefan Schillberg

Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Aachen, Germany

Prof. Dr. Stefan Schillberg is head of the Department for Plant Biotechnology and the division Molecular Biology at the Fraunhofer Institute for Molecular Biology and Applied Ecology (IME) in Aachen, Germany. He received his PhD in Molecular Biology from the RWTH Aachen University in 1994. Current activities in his laboratory focus on recombinant protein production in various host systems, downstream processing and recombinant protein analytics as well as metabolic pathway engineering and the analysis of molecular factors affecting cell productivity. He holds an honorary professorship at the Justus-Liebig-University in Giessen.

Abstract

Production of valuable compounds using plants and plant cells

Many different plant-based systems have been used to produce valuable polymers, small molecules or recombinant proteins but only a small number have made the leap from an experimental platform to a viable commercial process. This reflects a combination of factors, principally the technical issues that must be addressed to achieve competitive performance, the economic principles that need to be satisfied to ensure manufacturing processes are financially viable and sustainable, and the regulatory demands that must be met to ensure that products manufactured in plants are safe, efficacious and meet the quality standards demanded by the regulators. In general, plants and plant cells are ideal production platforms, which can be easily scaled up to provide valuable products for pharmaceutical, cosmetic and industrial applications.

In this presentation, the advantages and disadvantages of commonly used plant-based recombinant protein production platforms are presented as well as strategies for recovering the final product, for scale up and GMP-compliant production.

In addition, developments to use plant suspension cells for cosmetic applications are presented as well as strategies to increase plant biomass yield by enhancing the photosynthetic carbon fixation.

Ralf Reski

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Ralf Reski studied Biology, Chemistry and Pedagogics in Giessen and in Hamburg. He received his PhD in Genetics in Hamburg in 1990 and was appointed Assistant Professor in Cell Biology thereafter. Four years later, he was awarded his Habilitation in General Botany. From 1996 until 1999 he was a Heisenberg-Fellow of the German Research Foundation DFG. After offers from national and international Universities he was appointed Distinguished Professor of Plant Biotechnology at the University of Freiburg in 1999. He is also affiliated to École supérieure de biotechnologie Strasbourg (ESBS). Since 2011, Reski is Senior Fellow of the Freiburg Institute for Advanced Studies (FRIAS) and since 2013 also at the University of Strasbourg Institute for Advanced Studies (USIAS). Reski is founding PI of the Spemann Graduate School of Biology and Medicine (SGBM) and the Centre for Biological Signalling Studies (BIOSS). SGBM, BIOSS and FRIAS are funded by the German Excellence Initiative. USIAS is funded by the French Excellence Initiative.

Abstract

Moss: from basic biology and biotechnology to medical application

While most plant biologists concentrated on Arabidopsis research, we have developed the moss *Physcomitrella patens* from scratch to a model species in basic research and in biotechnology. The moss homologous recombination machinery allows the as yet unmatched precise and efficient genome engineering and gene identification by reverse genetics. This was used in a large-scale industrial cooperation to identify novel genes with implications for agriculture and human health. The Physcomitrella genome was fully sequenced as first genome of a non-vascular plant and as third plant genome after Arabidopsis and poplar. These resources are increasingly used by the plant community for evo-devo studies and led to the selection of the moss genome as one out of seven plant flagship genomes by the US Department of Energy, Joint Genome Institute.

Two other milestones from my lab are the first functional identification of an organelle-division protein in eukaryotes and the first description of microRNA-mediated gene silencing. This novel mechanism of eukaryotic gene regulation is increasingly discussed in the context of epigenetics and human diseases like cancer. In parallel, I founded a company in 1999 which is devoted to moss molecular pharming. A certified GMP production in moss bioreactors, successful upscaling to 500 L wave reactors, excellent homogeneity of protein glycosylation, remarkable batch-to-batch stability, and a safe cryopreservation for Master Cell Banking are some of the key features of the moss system. Several human proteins are being produced in this system as potential biopharmaceuticals. Among the products are tumour-directed monoclonal antibodies with enhanced antibody-dependent cytotoxicity (ADCC), vascular endothelial growth factor (VEGF), complement factor H (FH), keratinocyte growth factor (FGF7/KGF), epidermal growth factor (EGF), hepatocyte growth factor (HGF), asialo-erythropoietin (asialo-EPO), alpha-galactosidase (aGal) and beta-glucocerebrosidase (GBA). Further, an Env-derived multi-epitope HIV protein as a candidate vaccine was produced. Some of the recombinant biopharmaceuticals from moss bioreactors are not only similar to those produced in mammalian systems such as CHO cells, but are of superior quality (biobetters). The first moss-made pharmaceutical, aGal to treat Morbus Fabry, is in clinical trials to evaluate if the moss system is able to provide next generation biopharmaceuticals with superior quality over conventional mammalian systems.

Claudia Steinert

Ludwig-Maximilians-Universität München, Germany

Claudia Steinert works as a science journalist and mainly writes about ecology and medicine. Before starting her freelance career, she studied biochemistry at the University of Leipzig and went on to work at the Max Planck Institute of Molecular Plant Physiology (MPI-MP) in the Public Relations department. She is currently studying to obtain her master's degree in journalism at the Ludwig Maximilians University in Munich and the German School of Journalism.

Claudia Steinert will be moderating the discussion session "*The future of science and the future of me*".

Poster Abstracts

P 01| Pushing and pulling additional methionine into rice seed

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Rice is a staple food for approximately 3 billion people and can account for a significant proportion of their dietary protein, especially in the developing nations of Asia. However, rice protein is deficient in several amino acids essential in diets of non-ruminant animals, including methionine. Unless the diet is supplemented with other protein sources, these amino acid deficiencies can result in significant health consequences for humans and significantly stifled growth in animals. Previous work has shown that neither “pushing” methionine into rice seeds by increased amino acid synthesis nor “pulling” methionine into the seed by targeted expression of methionine-rich proteins, on their own, lead to appreciable improvements in seed protein quality. Here we show that combining “push” and “pull” modifications in the same plant can increase seed methionine by 50%. In addition to the demonstrated utility of the “push plus pull” double transgenic approach to increase protein-incorporated methionine in rice, we anticipate that it may have wider utility in development of crops with greatly improved nutritive quality for human and animal consumption.

Hagan ND et al. (2003) Plant Journal 34(1): 1-11

Nguyen et al. (2012) Journal of Experimental Botany 63(16): 5991-6001

P 02| Development of a new selection system for chloroplast transformation

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Plant transformation protocols generally involve the use of selectable marker genes for the screening of transgenic material. Several selectable markers have been developed over the last two decades and a number of traits have been used for selection. The most widely used and most efficient markers are isolated from bacterial genes that inactivate antibiotics. Several selection systems for chloroplast transformation are available that are based on resistance to aminoglycoside antibiotics, but with the exception of the spectinomycin selection system, they are neither efficient nor user friendly. Furthermore, none of them can be used for monocot transformation.

In this study, we report on the development of a new dominant selection marker for plastid transformation. We show that, by selecting for tobramycin resistance, tobacco chloroplast transformants are obtained at high frequency. Several transplastomic lines reach the homoplasmic state already during primary selection, while the

remaining lines require just one additional regeneration round. No spontaneous antibiotic resistance mutants appear upon tobramycin selection.

This selection system may be promising for future use in other species, including monocots.

P 03| A theophylline-responsive riboswitch as a novel tool for conditional repression of essential plastid genes in higher plants

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The plastid genome of higher plants contains several essential genes that are not open to functional analysis by targeted inactivation. One such gene, *accD*, codes for a plastid protein that is involved in *de novo* fatty acid synthesis in plant cells (Kode *et al.* 2005). Since *accD* is an essential gene, little is known about the consequences of its inactivation. Here we explore these consequences through the use of a riboswitch, which provides a novel tool for reverse genetics.

A riboswitch facilitates the functional analysis of essential genes by removal of the inducer, a binding ligand, which activates gene expression. We employed a theophylline-dependent riboswitch (Emadpour *et al.* 2015; Verhounig *et al.* 2010) to regulate translation initiation of *accD* in tobacco, by replacing its native 5' untranslated region (5' UTR) by the riboswitch. The specific chimeric phenotype of the mutant in the absence of theophylline allowed us to study the consequences of the AccD deficiency. The phenotype was characterized by periclinally chimeric leaves displaying deficient pigmentation especially in the L2 layer (leaf margin). In cells with strong repression of fatty acid biosynthesis, the levels of the chloroplast membrane-localized galactolipids were reduced and chloroplast division was impaired, tentatively explaining the pigment deficiency and impaired photosynthesis. By applying theophylline, we were able to rescue the mutant phenotype, due to induction of *accD* translation. This was confirmed at the protein level and by demonstrating recovery of plastid galactolipid levels.

Our results demonstrate that the theophylline-responsive riboswitch is able to provide dose-dependent on/off regulation of an essential protein. This valuable tool can be used to facilitate the functional analysis of other essential plastid genes for which currently no mutants are available.

Emadpour M, Karcher D, Bock R (2015) Boosting riboswitch efficiency by RNA amplification. Nucleic Acids Res 43, e66.

Kode V, Mudd EA, Iamtham S, Day A (2005) The tobacco plastid accD gene is essential and is required for leaf development. Plant J 44, 237-244.

Verhounig A, Karcher D, Bock R (2010) Inducible gene expression from the plastid genome by a synthetic riboswitch. Proc Natl Acad Sci U S A 107, 6204-6209.

P 04| Study of the Clp protease complex: a new strategy to identify protease substrates

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Protein degradation in the chloroplast is carried out by a set of proteases which are in charge of degrading misfolded, damaged or superfluous proteins. More than fifteen types of proteases are found in plastids, with Clp, FtsH, Lon and Deg being the most important (major) proteases. The ATP-dependent caseinolytic protease (Clp) is the most complex protease and it has been implicated in stromal protein degradation. In bacteria, the Clp protease is involved in N-end rule degradation suggesting an analogous role for this protease in chloroplasts.

To determine the effects of down-regulation of specific Clp subunits on plant growth and development and to identify possible Clp targets, constitutive and ethanol-inducible RNAi lines against different Clp subunits were generated in tobacco. Constitutively down-regulated lines displayed a wide spectrum of phenotypes, including pigment deficiency, alterations in leaf development, leaf variegation and impaired photosynthesis. To better distinguish primary from secondary effects and to be able to determine time-resolved changes in proteins stability, proteomic experiments were performed with the inducible RNAi lines. Time-resolved proteomic analyses will further allow us to identify new substrates of the Clp protease.

This work will provide first insights into the role of specific subunits of the Clp protease complex in protein degradation in tobacco plastids, and also will reveal target proteins of this protease.

P 05| Overexpression of serine-glyoxylate aminotransferase (SGT) increases the cellular glycine-to-serine ratio and decreases photosynthesis and plant growth

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During the last years photorespiration has emerged as valuable entry to optimize photosynthesis and plant growth. Therefor, different approaches were undertaken in order to either, reduce or circumvent photorespiration or to improve the internal metabolite conversion by up-regulating participating enzyme activities. Here we have generated and characterized *Arabidopsis thaliana* mutants overexpressing the peroxisomal serine-glyoxylate aminotransferase (SGT). The transgenic lines showed

considerably elevated SGT activity, mainly decreasing the leaf steady-state content of serine that in turn, increases the cellular glycine-to-serine ratio. However, at the phenotypic level transgenic plants are of indistinguishable size compared to the wild type but with significantly reduced numbers of rosette leaves, fresh weight and total biomass accumulation. Moreover, transgenic lines displayed slightly lowered rates of photosynthetic carbon assimilation in normal air which is pronounced with elevated photorespiratory pressure. In summary, our results provide evidence that SGT activity is not limiting during photorespiration, but nevertheless, the total SGT activity needs to be highly controlled while its overexpression negatively affects photosynthesis and plant growth. We hypothesize that this effect is caused by perturbation of the photorespiratory glycine-to-serine ratio and thus highlights the importance to accurately control the abundance of both amino acids.

Timm *et al.* (2012) Glycine decarboxylase controls photosynthesis and plant growth. FEBS Letters 586: 3692-3697

Sommerville and Ogren (1980) Photorespiration mutants of Arabidopsis thaliana deficient in serine:glyoxylate aminotransferase. PNAS USA 77: 2684-2687

P 06| Investigation of Rubisco – carbonic anhydrase fusions in tobacco as an approach to reduce photorespiration

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The initial step of carbon assimilation in C_3 plants is the carboxylation of ribulose-1,5-bisphosphate (RuBP) by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). Alternatively, Rubisco can catalyze the oxygenation of RuBP as the first step in photorespiration. This reaction is considered wasteful, since energy is needed to recover RuBP and CO_2 is lost in the process.

In order to compensate Rubisco's inability to differentiate between CO_2 and O_2, different mechanisms have evolved to raise CO_2 concentration in the surrounding of the Rubisco, such as C_4 metabolism or various forms of carbon concentrating mechanisms (CCMs) in microalgae and cyanobacteria.

With the same aim of increasing CO_2 at the site of the Rubisco, we have designed a set of new proteins that consist of fusions of the big subunit of Rubisco (RbcL) to a carbonic anhydrase that catalyzes in the interconversion of HCO_3 and CO_2.

We substituted the endogenous RbcL with these new proteins in *Nicotiana tabacum* through chloroplast transformation and analyzed the performance of the resulting plants. Initial results show that the plants are impaired in growth but retain the ability to grow autotrophically at ambient CO_2 concentrations.

This work represents the first evidence of a functional fusion to RbcL and is key to future steps in Rubisco engineering and photosynthesis improvement.

P 07| The cyanobacterial glycerate pathway as a peroxisomal bypass for plant photorespiration

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Recent results indicated that influencing the photorespiratory metabolism also affects photosynthesis. Hence, approaches modifying photorespiratory fluxes will potentially improve plant performance. While plants only perform the photorespiratory C_2-cycle, cyanobacteria possess two alternative routes for the conversion of photorespiratory intermediates – the oxidation of glyoxylate to CO_2 and the glycerate pathway. The latter route converts glyoxylate to glycerate via tartronic semialdehyde. These reactions are catalysed by glyoxylate carboligase and tartronic semialdehyde reductase.

In this work, the genes coding for these enzymes in the cyanobacterium *Synechocystis* sp. PCC 6803 were expressed in *Arabidopsis thaliana* Col-0 plants or Δ*hpr*1/Δ*hpr*2 double mutants. Since the C_2-cycle produces glyoxylate in the peroxisomes, the coding sequences of these genes were fused to a peroxisomal targeting signal to enable the transport of the proteins into this compartment. The expression of the cyanobacterial genes for the glycerate pathway was verified on transcriptional and translational level. Phenotypic and physiological analysis of transgenic plants showed that only a partial complementation of the Δ*hpr*1/Δ*hpr*2 mutations occurred. When grown under high CO_2 conditions slight growth promotion and improvement in CO_2-uptake ability was observed, but the plants retained the high-CO_2-requiring phenotype of the Δ*hpr*1/Δ*hpr*2 double mutants. The Col-0 transformands showed impaired growth and photosynthesis.

These results suggest that the expression of the genes for the glycerate pathway changed the photorespiratory flux and resulted in partial removal of glyoxylate. However, the changed flux in Col-0 plants negatively affects the interplay of photorespiration and other cellular processes.

Acknowledgements: We thank Klaudia Michl, Stefan Timm and Axel Masloboy for valuable help during this study.

P 08| The role of Ci uptake systems in the carbon concentrating mechanism

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In phototrophic organisms Ci-fixation is catalyzed by ribulose-1,5-bisphosphate carboxylase/oxygenase (RubisCO). This reaction competes with an oxygenation reaction that produces the toxic 2-phosphoglycolate (2PG). To avoid 2PG accumulation, cyanobacteria developed a CO_2 concentrating mechanism (CCM). The CCM consists of two components, the carboxysomes and high-affinity CO_2 or bicarbonate (HCO_3^-) uptake systems. The cyanobacterium *Synechocystis sp.* PCC 6803 (*Synechocystis* 6803) has five CO_2/HCO_3^- uptake systems: BCT1, a high-affinity HCO_3^- transporter, inducible by limited CO_2 availability, SbtA, an inducible high-affinity Na^+/HCO_3^- symporter, BicA, a low-affinity Na^+-dependent HCO_3^- transporter, NDH-1_4, a constitutive low-affinity CO_2 uptake system, and NDH-1_3, an inducible and high-affinity CO_2 uptake system. The uptake of CO_2 and HCO_3^- as major Ci sources as well as central carbon metabolism is tightly regulated in *Synechocystis* 6803.

We performed an integrated metabolic and transcriptomic analysis of a *Synechocystis* 6803 mutant defective in four CO_2/HCO_3^- uptake systems, Δ*ndhD3/ndhD4/cmpA/sbtA* (Δ4), shifted from high carbon (5% CO_2, HC) to low carbon (ambient air, LC) and implemented a qualitative comparison to previously published data characterizing a mutant lacking the central regulator of Ci assimilation Δ*ndhR* (Klähn et al., 2015) and a carboxysome-less mutant Δ*ccmM* (Hackenberg et al., 2012). The analysis revealed a strong intracellular Ci starvation phenotype of the Δ4 mutant even under conditions of sufficient Ci supply implying an intracellular physiological state of the Δ*4* mutant under HC conditions similar to the Ci-limitation of the *Synechocystis* 6803 wild type under LC conditions.

Hackenberg, C. et al. (2012) Low-carbon acclimation in carboxysome-less and photorespiratory mutants of the cyanobacterium *Synechocystis sp.* strain PCC 6803. *Microbiology* 158: 398–413

Klähn, S. et al. (2015) Integrated transcriptomic and metabolomic characterization of the low-carbon response using an *ndhR* mutant of *Synechocystis sp.* PCC 6803. *Plant Physiology* pp.114.254045

P 09| Should I stay or should I go? Sucrose breakdown appears to be an important mechanism during light-induced stomatal opening

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The control of stomatal aperture involves reversible changes in the concentration of ions and metabolites in guard cells. Sucrose has been proposed to act as an osmolyte during stomatal opening. However, recent evidences suggest that sucrose may perform other roles in guard cells in addition to the role as osmoticum.

Here we analyzed metabolic changes in guard cell enriched epidermal fragments from tobacco during light induced stomatal opening and characterized transgenic tobacco plants overexpressing sucrose synthase 3 (SUS3) under control of the stomatal-specific KST1 promoter.

Reduction in sucrose content was observed during light- plus- potassium induced stomatal opening. Concomitant with the decrease in sucrose we observed an increase in the level as well as in the ^{13}C-enrichment in metabolites of, or associated with, the TCA cycle. This experiment also provided a qualitative demonstration that CO_2 fixation occurs both via RubisCO and PEPcase in guard cells. Guard cell specific SUS3 overexpression led to increased stomatal conductance, transpiration rate, net photosynthetic rate, and growth. Whilst only minor changes were observed in the metabolite profiling in whole leaves an increased fructose level and decreased organic acid levels and sucrose to fructose ratio were observed in guard cells of transgenic lines.

Collectively the results suggest that sucrose breakdown would be a mechanism to provide substrate for the provision of organic acids for respiration and imply that manipulation of guard cell metabolism may represent an effective strategy for plant growth improvement.

Daloso DM et al. (2015) Plant, Cell & Environment *in press*

P 10| The role of trehalose-6-phosphate in plant metabolism and development

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In plants trehalose is synthesized via a phosphorylated intermediate - trehalose 6-phosphate (Tre6P). Tre6P has a large influence on plant metabolism, growth, and development. It has been demonstrated that Tre6P acts as a specific signal of sucrose availability. Tre6P is also proposed to act as a negative feedback regulator of sucrose levels, helping to keep these close to the optimum level for a given cell or tissue type.

Here, we introduce a cell- and tissue-specific system for inducible expression of heterologous Tre6P synthases (TPS) and Tre6P phosphatases (TPP), and use it to investigate cell- and tissue-specific mechanisms of Tre6P signaling in Arabidopsis.

Earlier studies have shown that sucrose plays a critical role in triggering bud outgrowth of decapitated pea plants. We measured metabolites in axillary buds of decapitated pea (*Pisum sativum* cv. Torsdag) plants, and observed that Tre6P starts to rise in the second axillary bud within 3 hours of decapitation, indicating a potential role for Tre6P in the sucrose driven initiation of bud outgrowth. Following this clue, we studied Arabidopsis mutants with constitutive or tissue-specific changes in Tre6P content, finding plants with high Tre6P, from over-expression of TPS, to have increased shoot branching and reduced apical dominance, whereas lowering Tre6P by over-expression of TPP has the opposite effects.

P 11| Metabolic characterisation of Arabidopsis early flowering 3 (elf3) mutants implicates the circadian clock's evening complex in the timing of starch degradation

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ELF3 is a major component of the circadian clock's evening complex (EC), which represses hypocotyl growth at the beginning of the night, acting indirectly via PHYTOCHROME INTERACTING FACTOR (PIF) proteins.

Metabolic traits and transcript abundance of core clock genes were analysed in the Arabidopsis *elf3-4* mutant and compared with the wild-type Ws2 background over a 24-h light-dark cycle, to explore the repercussions when the evening phase of the clock is impaired.

The *elf3-4* mutant showed several traits that were reminiscent of shade-grown plants: (i) a longer hypocotyl, (ii) smaller biomass and (iii) a lower chlorophyll a/b ratio compared to Ws2. The mutant has lower rates of starch accumulation and degradation and a starch excess at the end of the night. However, the levels of sugars during the day are higher in the mutant and drop suddenly at night. The peaks in expression of most of the clock genes are attenuated and/or delayed in the mutant.

Together, these data indicate that the action of the EC at dusk and at night influences clock function during the light period of the following day, and has a profound influence on carbohydrate metabolism.

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P 12| Unravelling 2-deoxy-2-fluoro-D-glucose metabolism in plant tissue using mass spectrometry and NMR

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2-Deoxy-2-Fluoro-D-glucose (FDG) is a structural glucose analogue which is commonly used as a radioactive glucose surrogate in clinical diagnostics and animal studies to trace uptake and metabolism of glucose in metabolically active tissue such as brain tissue or cancer cells. It mimics the glucose distribution and it was assumed that after uptake, it is metabolized via glycolysis pathway to FDG-6-phosphate but not further[1]. However, numerous papers describe the fate of FDG to FDG-6-P and further metabolites in the animal cells [2]. FDG has also been employed in plant radiotracer studies but its metabolism in plant cells is not yet characterized. Elucidating FDG metabolism in plants is a crucial aspect for establishing its application as a radiotracer in plant imaging. Here, we describe the metabolic fate of FDG in model plant species, *Arabidopsis thaliana*. We applied FDG to *Arabidopsis* leaf and analyzed leaf extract for fluorine (^{19}F) metabolites using MS and NMR. We demonstrate that FDG metabolism in plant cells is considerably different than animal cells and goes beyond FDG-6-P.

Mature leaves of *A. thaliana* (short day plants, 6-7 week, early flowering stage) were gently pricked on the abaxial surface. Five microliter of FDG (20 mg.mL^{-1}) was immediately applied in the pricked spots. Four hours later leaves were extracted using Chloroform:Methanol:Water (1:2:1). Aqueous fraction was analyzed by LC-MS/MS and NMR for the presence of ^{19}F-containing compounds.

LCMS and direct infusion MS results confirmed the presence of 5 different ^{19}F containing metabolites in the extract. In total, we putatively identified above ^{19}F containing metabolites as FDG (*m/z* 181.0513), F-gluconic acid (*m/z* 197.0464), FDG-6-P (*m/z* 261.0180), F-maltose (*m/z* 343.1051), and UDP-FDG (*m/z* 567.0434) on the basis of known literature information, their exact mono-isotopic mass (± 5 ppm mass error) and MS/MS fragmentation analysis. Characterization of purified compounds using NMR confirmed identification of ^{19}FDG-6-P (*m/z* 261.0180), and ^{19}F-maltose (*m/z* 343.1051) as major end products of ^{19}FDG metabolism in *A. thaliana* leaf cells.

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P 13| Identification of regulatory genes involved in central metabolism using Genome Wide Association and Knock-Out analysis.

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Enzymes constitute the molecular machinery for primary carbon (C) and nitrogen (N) metabolism, which provides the building blocks for growth. Previous studies in Arabidopsis populations showed that enzyme activities correlate with each other, suggesting that their levels are under tight common regulatory control (1, 2, 3). Analysis of genetic diversity for enzyme activities offers a powerful approach to identify genes underlying the metabolism regulatory network. Few QTLs co-localising with enzyme structural genes have been identified for trait variation (2). However, *trans* regulation remains largely unclear.

We performed Genome Wide Association (GWA) analyses on 360 Arabidopsis accessions for 21 enzyme activities and 13 metabolites from C and N primary metabolism in two independent experiments. A detailed comparison between the two datasets allowed us to select SNPs with high LOD-score in both experiments. GWA validation was carried out using 77 TDNA-insertion lines.

We cross-mapped the coarse QTLs found in the biparental population for the structural genes *UGP1* and *ATBETAFRUCT4*. In addition, high-LOD score SNPs were located in *trans* to enzyme structural genes. A total of 27 *trans*-regulatory QTLs were found, including a QTL associated to 4 enzyme activities, 3 metabolites, total protein and fresh weight. The metabolic phenotype of TDNA-insertion lines confirmed and validated *cis* and *trans*-regulation for a number of genes.

This study provides the highest defined QTL dataset for enzyme variation to date and breaks new ground in understanding the genetic regulation of central metabolism.

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P 14| Regulation of environmental stress memory in Arabidopsis through the AGO1-miR156-SPL module

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We present molecular and physiological evidence for the involvement of the microRNA pathway in heat stress memory. Different miRNAs are induced by high temperatures in Arabidopsis seedlings, and elevated expression is maintained for several days.

The miR156 family displays a particularly interesting expression profile of high induction and long maintenance, and we show its functional requirement by manipulating the levels of mature miR156. Depletion of miR156 increases damage after recurring heat stress, while its overexpression prolongs expression of heat stress memory genes and improves survival.

We furthermore show that repression of SPL genes, prominent targets of miR156 regulating the juvenile-to-adult phase transition, is required for heat stress memory. SPL genes are posttranscriptionally downregulated after heat, and this repression is a direct effect of miR156. Seedlings expressing miR156-resistant SPL transcripts are more susceptible to recurring heat stress and show a level of damage similar to that of miR156 knockdown lines. Defective heat stress memory in rSPL plants corresponds to reduced maintenance of heat stress memory genes, arguing for a role of SPL proteins as transcriptional repressors of these memory genes.

Altogether, our analyses demonstrate a yet unknown, central role of the miR156-SPL module in the integration of development and environmental stress.

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Stief A, Brzezinka K, Lämke J, Bäurle I (2014). Epigenetic responses to heat stress at different time scales and the involvement of short RNAs. Plant Signaling & Behavior, 9:10, e97043.

P 15| CIE is a novel transcription factor of *Arabidopsis thaliana* required for cold acclimation

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The Arabidopsis ethylene-response factor genes (*ERFs*) form a large gene family encoding plant-specific transcription factors. *ERF*s are involved in regulating numerous developmental processes. They are also important for adaption to various biotic and abiotic stresses. In this work, we report the identification of an AP2/ERF domain-containing transcription factor, named here *COLD-STRESS-INDUCED ERF* (*CIE*), which has a particularly relevant function in cold stress. Expression analyses revealed that *CIE* is early and transiently upregulated in cold within 1 h. Freezing experiments, e.g. electrolyte leakage tests, with loss-of-function as well as gain-of-function (overexpressing) mutants of *CIE* showed an altered freezing tolerance. The loss-of-function of *CIE* leads to less freezing tolerance whereas constitutive expression of *CIE* conferred freezing tolerance.

Expression levels of selected cold-responsive genes were investigated in *CIE* mutants before (non-acclimated (NA)) and after 14 d (ACC14) or 21 d (ACC21) of cold acclimation at 4 °C by quantitative RT-PCR. Consistent with the freezing phenotype, *cie* mutants showed a decreased expression of most cold-responsive genes, such as *CBF1*, *CBF2* and *CBF3* and a couple of *COR* genes in non-acclimated as well as in acclimated plants. In contrast, overexpression of *CIE* resulted in increased expression levels of those genes.

Further work showed that expression levels of genes of flavonoid and anthocyanin biosynthesis are altered in non-acclimated and acclimated *CIE* mutants as well.

Moreover, leaf proline, sugar (Glc, Frc, Suc, Raf) and anthocyanin contents of non-acclimated and acclimated *CIE* mutants were determined, but do not correlate with freezing phenotypes.

Taken together, we found a novel cold-regulated *ERF* transcription factor, that probably acts upstream of known cold-responsive genes. *CIE* positively regulates *Arabidopsis* cold acclimation and cold tolerance, respectively. However, its specific function during these processes still needs to be analyzed.

P 16| Effect of oxidative stress on salt-sensitive 'KDML 105' and salt-tolerant 'UBN 02123-50R-B-2' rice *Oryza sativa L.* seedlings

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Due to unavoidable adverse environments, plants are constantly exposed by various abiotic stresses. To retain growth and productivity, plants must have processes to adapt themselves in the changed environment. In this study, we compared the capacities of two five-day-old rice seedlings (*Oryza sativa* L.) cultivars, salt-sensitive KDML 105 and salt-tolerant UBN, under menadione-induced oxidative stress. The evaluation of oxidative stress by aconitase activity assay showed that the anti-oxidative processes in UBN were significantly better against the stress than in KDML 105 and were also correlated with our previous results concerning the better metabolic adjustment in UBN under salinity stress. Besides, we validated gene expression levels of some stress-responsive genes using quantitative real-time PCR and found higher induction levels of genes encoding glutamate decarboxylase and calmodulin 1-1 in UBN compared with those in KDML 105 under oxidative stress condition. Comparative metabolomics between these two cultivars and metabolite-to-gene correlation will be investigated further and would better provide a comprehensive understanding of plant adaptive responses to oxidative stress.

P 17| Natural variation in transcript abundance and derived cross-accession networks in *Arabidopsis thaliana* reveal a fingerprint of abiotic stress responses and glucosinolate metabolism

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Large-scale identification of transcripts with large cross-genotype differences in abundance provides a genome-wide approach to identify natural variation in gene function. Thus, we performed transcript profiling in 20 *Arabidopsis* accessions at dusk, dawn and after an extension of the night.

Individual genes with a high variance in transcript abundances across accessions were identified by inspection at each individual time point and by ANOVA across all three time points. Up to 40% of genes show significant changes in transcript abundance between accessions, with over-enrichment of e.g. biotic resistance, including pathogenesis resistance proteins and glucosinolate metabolism, and underrepresentation of photosynthesis, DNA synthesis, RNA processing, regulation of transcription, protein synthesis and protein targeting. Some functional classes were enriched at all three time points.

Cross-accession correlation networks were generated at each time point to identify sets of genes whose transcripts show coordinated change in abundance between accessions. A cluster that was highly enriched for cold-response genes was found at dusk and after an extension of the night. Another cluster found at all three time points was highly enriched for glucosinolate biosynthesis, and correlated with glucosinolate content. We conclude that genes involved in stress and defense related processes show especially large and coordinated natural variation in transcript abundance.

P 18| The *Pseudomonas syringae* type III effector HopZ1a targets a remorin possibly implicated in defense signaling

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Pseudomonas syringae uses a type III secretion system (T3SS) to deliver type III effector proteins (T3E) into host cells. These T3Es interfere with various immune responses to promote pathogenicity. HopZ1a, a member of the YopJ superfamily from *P. syringae*, was shown to display acetyltransferase activity towards tubulin leading to the inhibition of secretion during defense responses (Lee et al., 2012). To identify new HopZ1a targets we initiated a yeast-two-hybrid (Y2H) screen with a cDNA library from tobacco and HopZ1a as a bait protein. Our screen identified a remorin protein as a putative interaction partner of HopZ1a which we named HopZ1a interacting protein 1 (HIR1). To characterize a role of HIR1 in plant immune signaling we performed additional Y2H screens with HIR1 as a bait protein and identified PBS1, a protein kinase functioning in PAMP-triggered immunity (PTI), and SINA4, an E3 ubiquitin ligase as HIR1 interactors. Using split-YFP, we confirmed the interaction of HopZ1a and HIR1, as well as HIR1 and PBS1, with both complexes associating at the plasma membrane. Cell biological approaches revealed that upon flg22 treatment HIR1 shifts into punctuated structures at the plasma membrane, resembling lipid rafts and further suggesting a potential role of HIR1 during PTI. In summary, our findings support the hypothesis that HIR1 might act in a complex together with immune kinase PBS1 during PTI and hence is targeted by HopZ1a to manipulate immune signaling.

P 19| The impact of dark septate endophytes on plant nutrition

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Dark septate endophytes (DSE) are a group of ascomycetous fungi inhabiting the roots of many plants species in natural and anthropogenic ecosystems (Andrade et al. 2011a). Despite causing no obvious disease symptoms or symbiotic structures, they can have negative as well as positive effects on plant development and health (Andrade et al., 2011b). The need for better knowledge about DSEs as common root

colonizers has been addressed but nearly nothing is known about the mechanisms behind their effects.

In this study, the impact of two DSE species with known genome sequences on tomato was evaluated in the greenhouse under different nutritional conditions. The DSE species increased shoot biomass and this effect was more obvious on plant grown with available nitrogen and phosphorus in the soil. Furthermore, a compartment system was implemented, which was only accessible for fungal hyphae. Monitoring fungal spread and nitrogen and phosphorus analyses were carried out to test the hypothesis of DSE-mediated nutrient transfer from soil patches to plant roots.

P 20| Implementation of a simple tissue specific gatekeeper system allowing us to identify molecules moving between cells and tissues

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Plasmodesmata (Pd) are narrow, cytoplasmic channels that pass through plant cell walls. They provide a symplastic transport route for exchange of metabolites and macromolecules between cells and tissues. One of the few factors known to regulate Pd permeability is Callose (β-1,3-glucan) deposition at the neck region of Pd. Callose accumulates at Pd orifices and decreases the aperture of the Pd channel causing inhibition or complete blockage of cell-to-cell transport (1,2). To study the potential intercellular mobility of small metabolites and macromolecules we created a gatekeeper system based on the two callose modifying enzymes associated to Pd: Callose synthase (CALS3) and β-glucanase (BG-pap). The gatekeeper system employes tissue specific and inducible promoters enabling us to temporarily close or dilate Pd pores in in specific tissues such as the epidermis, mesophyll, and phloem in *A. thaliana*. To confirm the activity of the enzymes we used *CALS3:YFP* and *BG:YFP* fusion proteins and we performed callose stain and co-localization assays, and tested intercellular mobility. In addition, we are exploiting the gatekeeper system to study the developmental effects (phenotyping) and metabolite distribution after changing the symplasmic connectivity between various source and sink tissues in mutant and wild-type *A. thaliana* plants.

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P 21| The putative transcription factor STKR1: A novel component of SnRK1 mediated signaling?

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In plants, the Sucrose non-fermenting (SNF1)–related protein kinase 1 (SnRK1) represents a central integrator of low energy signaling and acclimation towards environmental stress responses. However, many components of SnRK1-regulated signaling pathways remain to be elusive. Recently, we have demonstrated that proteins containing a domain of unknown function (DUF) 581 interact with the catalytic alpha subunits of SnRK1 (AKIN10/11) from *Arabidopsis thaliana.* Arabidopsis possesses 19 DUF581 proteins, which consist of a variable N-terminal domain and a plant-specific highly conserved C-terminal DUF581 domain containing a novel type of C4-zinc finger that mediates the interaction with AKIN10/11. Bimolecular fluorescence complementation analysis confirmed interaction inside the plant nucleus.

Yeast-two hybrid analysis revealed several putative interaction partners of DUF581 proteins, including Storekeeper-related 1 (STKR1), a putative transcription factor that also interacts with AKIN10/11 in yeast and inside the nucleus of plant cells. Thus, we hypothesize that DUF581 proteins may modulate the interaction of SnRK1 and its potential targets such as STKR1. Interestingly, Arabidopsis plants overexpressing STKR1 show growth retardation and display a phenotype being reminiscent of plants overexpressing SnRK1, including anthocyanin accumulation and delayed senescence. These data suggest a possible involvement of STKR1 in SnRK1 mediated signaling.

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P 22| Small RNAs in chloroplasts and mitochondria represent footprints of RNA-binding proteins

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Regulatory small RNAs like miRNAs and siRNAs are well studied in plants. Using high-throughput sequencing small RNAs are usually sequenced from size fractionated total RNA. About 20% of sequence reads have an organellar origin and did not get a lot of attention so far.

We investigated small RNAs from a wide variety of plant species that map to the chloroplast and mitochondrial genome. We found about 100 of such small RNAs that show a strong bias towards localization in intergenic regions. We mapped transcript 5'- and 3'-ends and found that small RNAs and transcript ends coincide in most of the investigated cases. Whereas in chloroplasts, most 5'- and 3'-ends of mRNAs overlap with small RNAs, in mitochondria small RNAs are found to map mostly to 3'-ends of messages.

Several mRNA ends have been reported to be dependent on RNA-binding proteins and we find small RNAs with sequences that have been described as binding sites for RNA-binding proteins. Investigating "knock-outs" for RNA-binding proteins we show that processed mRNAs and small RNAs are missing in these mutants. This finding indicates that small RNAs might represent naturally occurring footprints of RNA-binding proteins.

Many of the known RNA-binding proteins that affect RNA stability in chloroplasts belong to the class of pentatricopeptide repeat proteins (PPR-proteins). This class of proteins has strong sequence specificity and members of this class bind with high affinity. Given the high specificity and affinity, it is speculated that these proteins act as protein caps preventing RNA degradation by Exonucleases (Pfalz J. et al. 2009). We show that sequencing of small RNAs in mutants of PPR proteins allows the identification of their target sites.

Pfalz J. et al. (2009) The EMBO Journal 28:2042–2052

P 23| Summarizing and exploring data of a decade of cytokinin-related transcriptomics

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The genome-wide transcriptional response of the model organism *Arabidopsis thaliana* to cytokinin has been investigated by different research groups as soon as large-scale transcriptomic techniques became affordable. Over the last 10 years many transcriptomic datasets related to cytokinin have been generated using different technological platforms, some of which are published only in databases, culminating in an RNA sequencing experiment. Two approaches have been made to establish a core set of cytokinin-regulated transcripts by meta-analysis of these datasets using different preferences regarding their selection.

Here we add another meta-analysis derived from an independent microarray platform (CATMA), combine all the meta-analyses available with RNAseq data in order to establish an advanced core set of cytokinin-regulated transcripts, and compare the results with the regulation of orthologous rice genes by cytokinin.

We discuss the functions of some of the less known cytokinin-regulated genes indicating areas deserving further research to explore cytokinin function. Finally, we investigate the promoters of the core set of cytokinin-induced genes for the abundance and distribution of known cytokinin-responsive *cis* elements and identify a set of novel candidate motifs.

P 24| Systems biology approach to lycophytes: *Selaginella mollendorffi*

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Expression atlas efforts, such as AtGenExpress for Arabidopsis, provide invaluable resource for a studied organism [1]. Currently, the common method used to determine the expression values of genes across tissue types and treatments has been microarrays. This method however has several disadvantages as the initial design of the array is expensive and often large amount of genes is absent from the microarray. However, recent advances within data analysis and sequencing have enabled the use of RNA sequencing (RNA-Seq) data to determine co-expression, with almost the same precision as microarrays [2].

We have generated an expression atlas for Selaginella moellendorfii, a model species for Lycophytes, and important ingredient of Chinese medicine,. It belongs to the first group of plants containing vascular tissue, and believed to have developed its root system independently,

The tissue atlas includes all major tissue types and environmental perturbations, such as cold, heat and time series. We have used the data to predict more than 8000 new transcripts. Held together with data from other evolutionary important species, this enables us to show how multiple pathways evolved and duplicated over time.

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P 25| From Dark to Light: the etioplast-to-chloroplast transition

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Proplastids, embryonic chloroplasts containing little or no membranes or proteins, can ultimately develop into chloroplasts, which contain the complex thylakoid membrane systems that house the protein complexes driving the light reactions of photosynthesis. In the absence of light, proplastids develop into etioplasts, containing non-photosynthetic membrane structures in the form of semi-crystalline prolamellar bodies. On lighting, etioplasts mature into functional chloroplast in the space of just hours, a feat requiring superb coordination of gene expression (from nuclear and plastid genomes), lipid and pigment synthesis, import of proteins, and insertion of proteins and cofactors in stoichiometric ratios into developing membrane systems. Perhaps unsurprisingly, many of the details of these processes remain unknown.

Here, we developed a novel system to investigate the time-resolved de-etiolation of a dicot leaf, and to date have explored and compared the accumulation kinetics of pigments, plastid-encoded transcripts, nuclear-encoded 'photosynthesis-related' transcripts, primary metabolites, and lipids, with future plans to measure

photosynthetic activity, and visualise chloroplast membrane structure. Currently, we are using an untargeted RNA-seq approach, and have short-listed new candidate genes, potentially involved in the etioplast-to-chloroplast transition.

P 26| A large-scale approach to study the mitochondrial proteome of *Arabidopsis thaliana*

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Proteins are involved in all aspects of live from enzymatic reactions to structural support. However, most enzymes are not active as monomer and most process in the cell are performed by multimeric protein complexes. So far, the most common approaches to study protein-protein interactions (PPIs) are biochemical purification followed by mass spectrometry, genetic engineering of cellular systems or microscopy techniques. These approaches have two disadvantages: they do not allow high throughput analysis (low number of PPIs can be studied per experiment), and a lack of quantitative data.

To avoid these shortfalls we use a large-scale approach to identify and quantify protein complexes. We focus our work on the mitochondrial proteome of *Arabidopsis thaliana*. The aim of the project is to compare the abundance and composition of organellar protein complexes under different environmental conditions or following in vitro treatments of isolated mitochondria. These experiments should provide insights into the regulation of plant metabolism on the level of protein complexes.

To achieve that purpose, native protein extracts of isolated and fully functional mitochondria are fractionated using native PAGE. Following electrophoresis the gel is cut in 24 fractions and in-gel digestion is performed to elute peptides. Samples are analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a Q-Exactive (Thermo Scientific) connected to a Easy n-LC II or 1000 (Thermo Scientific). Raw data is analyzed by Progenesis (Nonlinear DYNAMICS) and subcellular localization of identified proteins is obtained from the SUBA3 database (1). Protein complex formation is studied using in house created R scripts.

In an initial “proof of concept” study, quantitative data on 756 mitochondrial proteins was obtained. This equals coverage of 56% of the *A. thaliana* mitochondrial proteome. By comparing the theoretical molecular weight of the quantified proteins with their distribution profile in the native gel we calculated that approximately 60% these proteins are part of protein complexes. The data also reveals a clear tendency for the interaction between enzymes which catalyze neighboring reactions in metabolic pathways.

P 27| Metabolic and genetic variation in local populations of *A. thaliana*

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Plants are sessile organisms that must adapt to their local environment in order to survive. Natural variation in both local and global accessions of *Arabidopsis thaliana* can be used to understand mechanisms underlying adaptive plant responses. In this study we investigated the overall natural variation in two close populations, Altenriet (Alt) and Rübgarten (Rü) of *A. thaliana* collected in 2007, 2012 and 2013 around Tübingen, Southern Germany. Individuals of these populations were genotyped by RADseq. Using the 1985 informative SNP markers we first identified the genetic variation between and within the local populations of Alt and Rü. From AMOVA and haplotype analyses we were able to determine that the genetic variation is fluctuating between the selected years. Furthermore, we made diallel crosses among Alt individuals to study the overall variation in primary and secondary metabolites and how this relates to the genetic and phenotypic variation in this local population. Additionally, these diallel crosses allow the study of epistatic effects in this population. Moreover, we looked at the transcript levels of defense-related genes among the Alt population collected in the year 2007 and found variation in their expression profiles. These results suggest that natural variation is present even within one local population of *A. thaliana*. In the future, we will continue to unveil the role this diversity plays in the adaptation and evolution process of local plant populations.

P 28| Reproductive failure in *Arabidopsis thaliana* under transient carbohydrate limitation

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The impact of transient carbon depletion on reproductive growth in *Arabidopsis thaliana* was investigated by transferring long photoperiod-grown plants to darkness and returning them to a light-dark cycle. After two days of darkness, carbon reserves were depleted in reproductive sinks and RNA *in situ* hybridization of marker transcripts showed that carbon starvation responses had been initiated in the meristem, anthers and ovules. Treatments of two or more days resulted in a bare-segment phenotype on the floral stem, with 23-27 aborted siliques. These resulted

from impaired growth of very immature siliques, and abortion of mature and immature flowers. Depolarization of PIN1 protein and increased DII-VENUS expression pointed to rapid collapse of auxin gradients in the meristem and inhibition of primordia initiation. After transfer back to a light-dark cycle, flowers appeared and formed viable siliques and seeds. A similar phenotype was seen after transfer to sub-compensation point irradiance or CO_2. It also appeared in a milder form after a moderate decrease in irradiance, and developed spontaneously in short photoperiods. We conclude that *Arabidopsis thaliana* inhibits primordia initiation and aborts flowers and very young siliques in C-limited conditions. This curtails demand, safeguarding meristem function and allowing renewal of reproductive growth when carbon becomes available again.

P 29| F2 hybrid chlorosis in *Arabidopsis thaliana*

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Hybrid chlorosis is a type of hybrid incompatibility associated with reduced leaf chlorophyll content which can lead to stunted growth, yield loss or premature lethality. Therefore it is assumed to act as a postzygotic reproductive barrier in many crop species but mechanistic evidence is still missing.

We have identified a case of F2 hybrid chlorosis in the cross between two *A.thaliana* accessions, Shahdara (Sha) from Tajikistan and Lovvik-5 (Lov-5) from Sweden. The chlorotic F2 hybrids show reduced chlorophyll content accompanied by impaired photosynthesis, especially in immature leaves. These hybrids also produce less seeds in comparison to their parental lines but they do not show reduced growth.

The F2 segregation of chlorotic plants indicated that two recessive interacting loci are causing the phenotype. Illumina based mapping confirmed that a 200 kb interval at the beginning of chr1 (from Sha) and a two-fold larger interval on chr5 (from Lov-5) were linked to chlorosis. By comparing the genome sequence and doing additional crosses with genetically similar accessions, we first identified 13 candidate genes on chr1 and 24 genes on chr5. These genes were silenced separately in parental lines and in F3 lines where the phenotype is fixed using amiRNAs. On chr5, silencing of At5g05450 rescued the phenotype in the F3 lines. Furthermore, the rRNA profile showed an imbalance of the 18S/25S rRNA ratio indicating a possible role of At5g05450 in rRNA processing.

We are currently analyzing the link between rRNA processing and F2 hybrid chlorosis in detail as well as confirming the candidate genes on chr 1.

P 30| Hybrid necrosis in local populations of *Arabidopsis thaliana*

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Hybrid necrosis, caused by an auto-activation of the defense system is a common type of post-zygotic hybrid incompatibility in plants. The genetic basis underlying hybrid necrosis in *Arabidopsis thaliana* involves interactions between regulators and receptors of the defense response. In our project, we are investigating cases of F1 hybrid necrosis identified among different local populations of *A. thaliana* collected from the Tübingen area, Germany, in 2007. Identified necrotic hybrids showed cell death, ROS accumulation, reduced cell size, leaf initiation rate and seed yield as well as higher levels of salicylic acid and upregulation of *PR1*. Using linkage analysis, amiRNA silencing and genomic complementation we found that allelic interactions of *ACD6* (*ACCELERATED CELL DEATH 6*) were necessary and sufficient for the necrotic phenotype. Further crosses of individuals from different Tübingen populations allowed us to identify altogether 27 phenotypically similar cases of hybrid necrosis among four populations. Moreover, the auto-activation of the defense response in these hybrids is temperature-dependent, necrotic lesions are only visible in temperature above 20°C. Therefore we compared the metabolic profiles of hybrids to parental lines at 17°C and 21°C from which we identified metabolites specific for the hybrids. Subsequently, we performed a detailed analysis of selected metabolic biosynthetic pathways in hybrids focusing on tryptophan-derived compounds. The knowledge gained from studying *ACD6* may be useful in further understanding how plants deal with pathogen attacks in nature and the different responses that underlie these host-pathogen interactions.

P 31| An extra-nuclear speciation barrier is conferred by Programmed Cell Death in evening primroses (*Oenothera*)

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The genus *Oenothera* is a classical model for chloroplast mediated speciation barriers, but incompatibilities have been described which are independent from the chloroplast and nuclear genome. This indicates the involvement of a further cytoplasmic component.

One of these incompatibilities is the "*falcifolia*-syndrome", leading to malformation of organs. It arises in reciprocal crosses between *O. glazioviana* and *O. biennis*, two species forming natural hybrids. Depending on the crossing direction, the phenotype

occurs with different frequency. This points to a biparental inheritance of an extra-nuclear determinant, likely the mitochondrion.

To address this question, we initiated comparative mitochondrial genome sequencing, based on highly pure mitochondrial DNA, obtained from leaf material. NGS data from 454 and Illumina HiSeq were assembled *de novo*, employing various assemblers (CLC, MIRA, Newbler, IDBA-UD). New assembly and scaffolding strategies allowed for the first time the challenging data interpretation and resulted in a putative three-dimensional branched *in vitro* structure of the mitochondrial genome. It could be accomplished with IDBA-UD, which is designed for metagenomic sequencing data. This theoretical structural model of the mitochondrial genome is confirmed *in vivo*.

Although genetic mapping of *falcifolia* populations could not link the phenotype to the mitochondria so far, histological analyses strongly suggest Programmed Cell Death as underlying mechanism causing organ aberration in the hybrids.

P 32| The psbB-operon is a major locus for plastome genome incompatibility in *Oenothera*

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The model plant *Oenothera* (evening primrose) is perfectly suited to study molecular cause and evolutionary driving forces, connected with cytoplasmic elements in speciation. Hybrid offspring within the genus often displays so-called plastome-genome incompatibility (PGI), a plastid mediated speciation barrier. Within the *Oenothera* genus three basic nuclear genomes (A, B and C), that can occur in either homozygous or stably heterozygous constitutions, and five distinguishable plastid chromosomes (I-V) were identified. All haploid nuclear genomes and plastome types are freely combinable in altogether 30 combinations. Only 12 of them are phenotypical green and only seven exist in nature. The remaining 18 combinations display PGI to various degrees and can occur naturally as inviable hybrids.

In a pilot study the incompatible combination AB-I was chosen to identify molecular determinants causing PGI in *Oenothera*. This incompatibility builds a strong, asymmetric hybridization barrier between AA-I and AB-II/AB-III species. From formal genetic data it appears, that plastome type I is incompatible in the AB background, but the combination AB-II, AB-III, and AB-IV remains green. Sequence comparison of these four plastomes unveils two appealing candidate loci specific for plastome I, but not present in plastomes II-IV: a deletion of 148 bp, and an insertion of 16 bp, both in intergenic regions between genes involved in the photosynthetic apparatus. Physiological and molecular analyses substantiate their relevance causing PGI in AB-I *Oenothera* plants. The deletion affects the promoter region of the *psbB* operon (*psbB-psbT-psbH-petB-petD*).. These genes encoded for subunits of photosystem II (PSII) and the cytochrome b_6f complex. The whole operon, together with *pbf1* transcribed from the antisense stand, is downregulated in AB-I incompatible material. The insertion, in turn, affects mRNA transcript stability/ turnover of the *petN* and *psbM* genes, both encoding for small weight subunits of cytochrome b_6f and PSII, respectively. The findings are in line with photosynthetic parameters and protein abundance. Thus difficulties of plastid transformation in *Oenothera*, our observations

were confirmed by heterologous combinatorial transformation of *Nicotiana tabacum*. In obtained homoplasmatic tobacco lines, TT→GG substitution in -35 element of *psbB* promoter (P*psbB*) *resulted* in plants with similar phenotype to AB-I hybrids. Physiological and molecular analysis of tobacco plants were well in line with examination of incompatible *Oenothera* hybrids, emphasizing importance of 148 bp deletion in *clpP*/*psbB* intergenic region in PGI.

P 33| The plastidic leaf shape mutant *pm45*

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Leaf development and shaping are largely enigmatic processes. Classic genetic evidence clearly shows an involvement of the chloroplast in these processes. The leaf shape mutant *pm45* derived from a *plastome mutator* mutagenesis performed on the green neutral mutant *Cornell-1*. To identify the polymorphism responsible for the leaf shape phenotype, *pm45* and *Cornell-1* plastomes were sequenced. Comparison between the two plastomes revealed a *pm45* specific frameshift in *ycf1*, multiple INDELs in the 5' end of *accD* and a single base pair insertion in *ndhD*. The first two genes have been shown to be essential in *Nicotiana* tabacum and have an important role in the cell formation, chloroplast function and maintenance. *accD* encodes for the β-carboxytransferase subunit of the Acetyl coA Carboxylase (ACCase) enzyme which involved in the first step of fatty acid biosynthesis. AccD is the only plastid encoded subunit of the enzyme. Lipids profile on *pm45* leaves shows a drastic decrease of the main classes of chloroplast membrane lipids, MGDG and DGDG, in comparison to *Cornell-1*. This result has also been observed in the *N. tabacum accD* knock down mutant which as well shows a leaf shape phenotype. The function of *ycf1* remains still unknown due to the lack of genetic evidence. In order to distinguished between the two candidate genes, *ycf1* and *accD*, and to study the function of Ycf1 protein, we are currently generating a *N. tabacum* mutant harboring a premature stop codon in the middle of *ycf1*.

P 34| Plastids alter leaf morphology in plastome-genome-incompatible *Oenothera* hybrids

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The plant genome comprises the nuclear genome, the plastid genome (plastome) and the mitochondrial genome. Nuclear-organellar interaction is crucial for plant development and function, consequently resulting in coevolution of the genetic compartments. Hence, new combinations of nuclear and cytoplasmic genomes can cause incompatibilities even between closely related species.

An interesting plastome-genome-incompatibility altering leaf morphology can be observed in certain interspecific *Oenothera* hybrids. Reciprocal crosses between *Oenothera berteriana* and *O. odorata* yield hybrids which have identical nuclear genomes combined with differing cytoplasms. Hybrids hosting *O. berteriana* plastids have green and broad leaves, whereas *O. odorata* plastids are incompatible with the hybrid nuclear genome and confer *virescent* (periodically pale) and narrow leaves. We uncovered 146 polymorphisms distinguishing *O. odorata* from *O. berteriana* plastomes, representing a broad range of loci potentially responsible for the observed leaf phenotype. An association mapping approach based on full plastome sequences of various *Oenothera* accessions helped narrow down the list to 46 putative candidates. We could exclude most of them by complementary molecular analyses, which rather point towards lipid biosynthesis to be involved in the plastome-genome-incompatibility and altered leaf morphology.

P 35| Getting back to nature: a reality check for experiments in controlled environments

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Plants growing in nature experience diurnal changes in irradiance and light quality, with gradual shifts between light and dark at dawn and dusk. In contrast, many experiments in controlled environment chambers expose plants to a constant irradiance during the day period and sharp transitions between light and dark. To investigate the physiological effects of different light regimes, we grew Arabidopsis Col-0 plants in a naturally illuminated greenhouse around the spring equinox, and harvested plants at intervals throughout the 24-h light-dark cycle to measure metabolites and the abundance of circadian clock gene transcripts. Results were compared with plants grown in controlled environment chambers with a similar 12-h photoperiod and daily light integral (7 $mol \cdot m^{-2} \cdot d^{-1}$) to the greenhouse conditions, but with either square-wave or sinusoidal artificial light. The levels and diurnal patterns of several metabolites differed significantly between the three growth conditions, especially around dawn and dusk. The timing of expression of clock “morning” genes was little affected by light regime, whereas “evening” gene expression patterns were significantly changed.

P 36| On the ecology of fear: biological control of four aphid genotypes under the different types of exposure to natural enemy.

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Green peach aphid (*Myzus persicae*, Sulzer) is a generalist phloem feeding insect and a noxious plant-virus vector. This aphid infests a wide range of natural and agricultural host plants and reacts with outstanding plasticity to a variety of environmental stimuli. Under preferable condition, *M. persicae* forms clones of genetically identical individuals through parthenogenesis which grow exponentially in numbers while congregating on vulnerable resources. One important factor that influences the ecology and response of this aphid is the relationship with its natural enemies. Similar to what is observed in a spectrum of other predator-prey systems, the presence and hence the perception of a predator in the vicinity of the aphid clone elicit a varied response spanning change in fecundity, production of morph, hiding within concealed parts of the host plant and abandoning risky spots. In this work we exposed each of a set of four *M. persicae* clones to distinct types of encounter with lacewing aphidophagous larvae. The treatments respectively included free aphid-chasing larvae, euthanised and plant-tethered predator, shoot-sprayed and below-ground infused solution of homogenised larvae. In contrast to the aphid control lines we found strong effects of the presence and absence of exposure to predator-threat on aphid reproductive success, choice for host plant and within-plant distribution. Moreover, we quantified the differential influence of predation threat on the focal aphid clones and found notable effects of aphid genotype, type of exposure to predator and their interaction on aphid fitness and behaviour. This work innovatively adds to the increasing evidence on the magnitude of the direct and indirect ecological effects of predation on the interaction between plant pests i.e. aphids and their natural enemies. Moreover, the findings of this study highlight the importance of the application of alternative aphid bio-control exercises that are easily integrable and utilisable in agroecosystems.

Notes

Notes

Plants and People conferences are held every two years. We began in 2011 with the inaugural ‘On Roots and Fruits of Plant Biology’, looking into the history and present day applications of plant research, while our 2013 conference ‘Challenges in Biology - Big Data and Ethics’, focussed on advances in biology and addressed ethical challenges.

We hope you enjoyed this year’s P&P conference

‘Future Plan[t]s’

Our next meeting will be in 2017.

We hope to see you then!

The 2015 organising team

Doctoral students of the MPI-MP (left to right):

Jana Dotzek, Iman Tabatabaei, Franziska Fichtner,

Tegan Armarego-Marriott, Mercedes Diez Cocero, Selin Bülbül,

Bjørn Øst Hansen, Isabel Orf, Tommaso Pellizzer

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