Plants and People Conference

MAX-PLANCK-GESELLSCHAFT

Challenges in Biology – Big Data and Ethics

Ethics and Good Scientific Practice

Current Progress in Plant Biology

18-19 June 2013

Max Planck Institute
of Molecular Plant Physiology
Potsdam, Germany

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

Organised by PhD students

IMPRS Primary Metabolism and Plant Growth

Max-Planck-Institut für Molekulare Pflanzenphysiologie

Universität Potsdam

ONLINE Version of the Plants and People 2013 Abstract Book

Welcome!

Dear colleagues and guests,

We are happy to welcome all participants of the “Plants and People Conference 2013” on the Max Planck Campus Golm. The theme of our second meeting is: “Challenges in Biology – Big Data and Ethics”. Speakers from across the globe will address a broad range of topics integrating physiological, genetic, molecular and bioinformatic aspects of life science research as well as the fundamental issues crucial to guiding us on the most ethical path possible in our research.

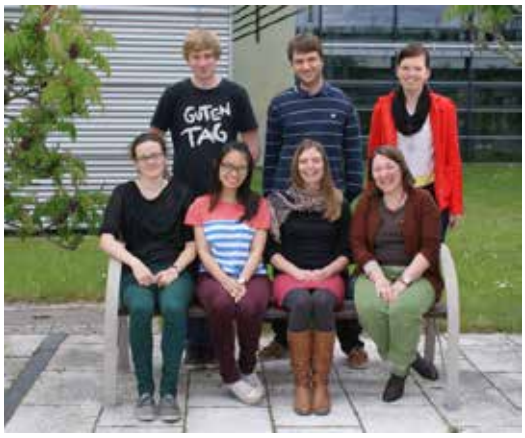
Our conference is hosted by the Max Planck Institute of Molecular Plant Physiology. The MPI-MP was founded in 1994 and has developed into one of the world’s leading plant research institutes. “Plants and People” aims to provide a platform for the participants to get together with other scientists and to discuss ideas and experiences. We hope that you, our delegates, will find this year's meeting just as interesting as our 2011 inaugural conference, and will leave at the end of the day armed with plenty of fresh new ideas to take back and put into action.

We wish you interesting and fruitful days with many discussions and new contacts!

The organising team

Doctoral students of the MPI-MP

Elmien Heyneke
Heike Sprenger
Jessica Jüppner
Maximilian Fünfgeld
Phuong Anh Pham
Vinzenz Hofferek



Thank you!

Elisa Schulz, Jörn Lämke and all helpers on the two conference days for support, Dr. Ina Talke and the administration of the MPI-MP for support in planning and organising, Marcus Thienert and Jan Scharein for web design, the 2011 P&P team for logo design and breaking the ground, and the Max Planck Society and IMPRS “Primary Metabolism and Plant Growth” for financial support.

Many thanks to Conviron, Roboklon, Perkin Elmer & NEB 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, 17.06.
16:00 – 18:00	**Early registration**

Time	Tuesday, 18.06.
09:00 – 09:45	**Registration**
09:45 – 10:45	**Welcome** ***Keynote:*** Susanne von Caemmerer (AUS)
10:45 – 11:00	**Coffee break**
	Plant Metabolites
11:00 – 11:45	Dirk Prüfer (D)
11:45 – 12:30	Andreas Weber (D)
12:30 – 13:30	**Lunch (not provided)**
	Making Sense of Omics
13:30 – 14:15	Sue Rhee (USA)
14:15 – 15:00	Eve Syrkin Wurtele (USA)
15:00 – 15:45	Manpreet Katari (USA)
15:45 – 16:15	**Coffee break**
	Ethics and Good Scientific Practice
16:15 – 17:00	Anne Ingeborg Myhr (NO)
17:00 – 17:45	André van Steirteghem (BE)
From 18:00	**Poster session and evening reception with BBQ**

Time	Wednesday, 19.06.
	Epigenetics
09:15 – 10:00	Anna Amtmann (UK)
10:00 – 10:45	Ortrun Mittelsten Scheid (AT)
10:45 – 11:15	**Coffee break**
	From Fluxes to Pathways
11:15 – 12:00	Louwrance Peter Wright (D)
12:00 – 12:45	Uwe Sauer (CH) / Hannes Link (CH)
12:45 – 14:00	**Lunch (not provided)**
	Signalling and Defence
14:00 – 14:45	Patrick van Dijck (BE)
14:45 – 15:30	Junji Yamaguchi (JP)
15:30 – 16:00	**Coffee break**
	Ethics in Science
16:00 – 16:45	Matin Qaim (D)
16:45 – 17:30	**Panel Discussion** moderated by Claudia Steinert
17:30 – 17:45	**Closing remarks**

Conference Venue

Max Planck Institute of Molecular Plant Physiology

The mission of the institute is to study plant metabolism in the context of the plant system as a whole. Since this system is more than a collection of genes and their products, we focus our efforts on understanding how these components dynamically interact over time and under different conditions.

To grasp the complexity, it is essential to determine molecular and complex parameters at different functional levels, and to analyse the resulting complex data set against the background of existing biological knowledge.

In the long run our goal is to develop a comprehensive, systems-level understanding of plant growth. To link plant growth and metabolism the institute maintains a strong interest in understanding the genetic and physiological basis of biomass formation and heterosis. Current efforts are centred on unravelling a set of complex processes that are of particular relevance to plant biomass: the bioenergetic pathways, macronutrient acquisition, nutrient signalling and resource allocation.

A detailed understanding of plant growth represents one of the greatest challenges in plant physiology. Our institute is very well prepared to accomplish this challenge.

To find out more about the Max Planck Institute of Molecular Plant Physiology, please visit our website: http://www.mpimp-golm.mpg.de/

Travel Information

Connections to Potsdam Hauptbahnhof (regional trains and the S-Bahn to/from Berlin):

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) to the train station Potsdam-Golm:

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 into RB 21 or RB 22.

It is a 5-10 min walk between the 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 next to 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

The profiles and abstracts of our invited speakers are given in the order of their talks during the meeting.

Susanne von Caemmerer

Research School of Biology, The Australian National University, Acton, Australia

Susanne von Caemmerer was born and grew up in Freiburg, West Germany. She moved to Australia in 1973 to commence studies at the Australian National University. There she completed undergraduate studies in pure mathematics in 1976 followed by a PhD in plant physiology in 1981. She is now Professor of Molecular Plant Physiology at the Research School of Biological Sciences at ANU. Her research focuses on photosynthesis, with an emphasis on the mathematical modeling of the carbon acquisition of plants, the biochemistry of carbon dioxide fixation and regulation of carbon dioxide diffusion in leaves.

Abstract

The development of C4 rice: current progress and future challenges

To meet the challenge of increasing crop yield for a burgeoning world population, it has become apparent that photosynthetic efficiency and capacity must be increased per unit leaf area to improve yield potential. High yields from C4 crops have stimulated considerable interest in the C4 photosynthetic pathway which is characterised by high photosynthetic rates, high nitrogen and water use efficiency relative to plants with the C3 photosynthetic pathway. The international C4 rice consortium is working towards introducing the C4 pathway, into rice to increase yield. The goal is to identify the genes necessary to install C4 photosynthesis in rice through a number of different approaches, including genomic and transcriptional sequence comparisons and mutant screening.

Dirk Prüfer

Westphalian Wilhelms-University Münster, Institute of Biochemistry and Biotechnology of Plants, Münster, Germany

Dirk Prüfer is full-professor for Plant Biotechnology at the Institute for Plant Biology and Biotechnology at the Westphalian Wilhelms-University Münster (WWU) and head of the branch office “Plant Biopolymers” of the Fraunhofer-Institute for Molecular Biology and Applied Ecology in Münster.
He studied Biology at the University of Cologne and performed his PhD research at the Max-Planck-Institute for Plant Breeding. His major research interests are in plant breeding, in particular in the improvement of plant architecture and metabolic pathways for polymers such as starch and rubber. At present, he is the Dean of the Faculty of Biology and speaker of the graduate school “Renewable Resources” at the WWU.

Abstract

Improved production and extraction of plant-derived polymers by genetic engineering and chemical mutagenesis

Biopolymers such as starch, cellulose and rubber are the most abundant organic compounds in the world and they are used to manufacture many technical products used in daily life. Unfortunately, the use of biopolymers is often neither environmentally nor financially sustainable. One of the aims of modern breeding programs is therefore to develop new traits that allow the sustainable and high-yielding production of improved and/or novel biopolymers in plants. These targeted improvements require a fundamental understanding the underlying biosynthesis pathways and the function of key enzymes. Functional genomics is a powerful tool to investigate such pathways, identify the functions of the corresponding genes and enzymes, and identify pathway bottlenecks that can be targeted for improvement to increase the production of both primary and secondary metabolites.
Our research includes projects that focus on the characterization and modification of starch biosynthesis in potato and rubber biosynthesis in the Russian dandelion as model systems. We have developed both genetic engineering and non-GM smart breeding strategies to improve the quality, quantity and extractability of natural rubber and high-amylopectin starch. To support the sustainable development of new traits and their exploitation throughout the value chain, these activities include the creation of new germplasm, the detailed analysis of product biochemistry and genetics, breeding, agronomy, processing and product development. We will present our recent achievements concerning the production of new traits in potato and Russian dandelion.

Andreas Weber

Center of Excellence on Plant Sciences (CEPLAS), Institute of Plant Biochemistry, Heinrich-Heine-University, Düsseldorf, Germany

Andreas PM Weber was born 1963 in Würzburg. He completed his doctorate in 1996 and habilitated in 2002 at the University of Cologne on "The plastid transporters as connecting elements between plastidic and cytosolic metabolism". Andreas Weber was Associate Professor of Plant Biology at Michigan State University-East Lansing (USA) from 2002 to 2007. He is now Professor at the Institute of Plant Biochemistry at the Heinrich-Heine-University in Düsseldorf. His research interest include the physiology, biochemistry and molecular biology of solute transport in plant cells; compartmentation of metabolic pathways and metabolic networks; photorespiration; C4 photosynthesis; extremophilic eukaryotes; 'Omics technologies and synthetic experimental evolution; synthetic biology.with the systems biology of the intracellular Metabolite transports. Andreas Weber is co-editor of the journals Plant Physiology and Plant Biology and he is Chairman of Physiology at & Molecular Biology of the German Botanical Society.

Abstract

Towards a molecular blueprint for engineering C_4 photosynthesis

C_4 photosynthesis involves alterations to the biochemistry, cell biology, and development of leaves. Together these modifications increase the efficiency of photosynthesis, and despite the apparent complexity of the pathway, it has evolved at least 45 times independently within the angiosperms. To provide insight into the extent to which gene expression is altered between C_3 and C_4 leaves, and to identify candidates associated with the C_4 pathway we used massively-parallel mRNA sequencing of closely related C_3 (*Cleome spinosa*) and C_4 (*Cleome gynandra*) species. This analysis shows that 818 transcripts differ in abundance between these C_3 and C_4 leaves. This includes twenty transcription factors, putative transport proteins, as well as genes that in *A. thaliana* are implicated in chloroplast movement and expansion, plasmodesmatal connectivity, and suberin deposition. These are all characteristics known to alter in a C_4 leaf, but which previously had remained undefined at the molecular level. Unexpectedly, we document a large reduction in transcripts encoding ribosomal subunits. Our approach defines the extent to which transcript abundance in these C_3 and C_4 leaves differs, provides a blueprint for the NAD-ME C_4 pathway operating in a dicotyledon, and furthermore identifies potential regulators. We anticipate that comparative transcriptomics of closely related species will provide real insight into the evolution of other complex traits.
In this presentation, I'll focus on the identification and functional analysis of metabolite transporters required for C_4 photosynthesis, with specific focus on transporters residing in the chloroplast envelope membrane.

Sue Rhee

Carnegie Institution for Science, Department of Plant Biology, Stanford, CA, USA

Seung Yon (Sue) Rhee received a B.A. from Swarthmore College and a Ph.D. from Stanford University. Her group studies how plants control their metabolism in response to their environments. Their main approach is to build genome-wide networks of metabolism, regulation, and signaling to understand how they function and evolve. Through this process, they are discovering novel components and pathways within these networks. They employ a wide array of tools including bioinformatics, computational biology, functional genomics, proteomics, metabolomics, genetics, molecular biology and biochemistry to address these questions.

Abstract

Genomic signatures of specialized metabolism in plants

Humans depend on plant metabolism for survival and well-being. For example, over 25% of drugs are natural products or derivatives of plant metabolism. Despite our dependence on plant metabolism, we know very little about it. Of the estimated 200,000-1,000,000 metabolites and >1 billion enzymes in plants, we know about how ~1000 metabolites are made by ~3000 enzymes. Therefore, plant metabolism is one of the most understudied areas in biology and medicine with a huge unrealized potential for discovering new chemistry and biology. We wish to understand how plants control their metabolism in response to environmental signals. We also want to understand why different plants have adapted different ways of responding to their environments. With these understandings, we expect to be able to engineer plants to optimize their metabolism under different environmental scenarios. Towards this end, we developed a high-quality prediction system for metabolic enzymes called E2P2 by integrating different prediction programs. Using E2P2, we generated and compared metabolic networks of *Arabidopsis thaliana, Carica papaya, Glycine max, Manihot esculenta, Physcomitrella patens, Populus trichocarpa, Selaginella moellendorffii, Vitis vinifera,* and *Zea mays* and 1 alga (*Chlamydomonas reinhardtii*) (www.plantcyc.org), which led to the discovery of several novel genomic signatures of specialized metabolism, which could be used to predict enzymes and pathways that make specialized metabolites. I will present and discuss our findings from this work.

Eve Syrkin Wurtele

Department of Genetics, Development and Cell Biology,
Iowa State University, Ames, IA, USA

Eve Syrkin Wurtele, Professor at Iowa State University, received a B.S. from UC-Santa Cruz, and a Ph.D. in Biology from UC Los Angeles. After a postdoctoral fellowship at UC-Davis and Senior Scientist at NPI, a biotechnology company, she joined Iowa State. Her research, juxtaposed at the interface between biological and computational sciences, centers on the interplay between metabolic and regulatory signals. The research is revealing a complex network that mediates accumulation of proteins, starches, oils, and specialized natural products. Prof. Dr. Wurtele also directs the award-winning computer game, Meta!Blast, which takes a player into an interactive metabolic adventure within a 3D photosynthetic cell.

Abstract

Analysis of metabolic and regulatory networks

Understanding of how plant composition is regulated has been elusive. The factors that regulate metabolism are key to utilization of crops for improved plant composition and production of novel constituents, yet little is known concerning the mechanisms controlling how much carbon flows to oil, starch, protein and other constituents. We identified a regulatory function in starch metabolism for Arabidopsis locus At3g30720 (QQS); transgenic lines with up- or down-regulated QQS expression have a normal appearance but an altered starch content [1], and a transcriptome with shifts in the accumulation of specific transcripts. The QQS gene is one of the many so-called orphan genes, it is unique to a single species. We are iteratively combining bioinformatics analyses and experimentation to expand our understanding of the network that mediates this compositional change.

[1] Li L, Foster CM, Gan Q, Nettleton D, James MG, Myers AM, Wurtele ES (2009) Identification of the novel protein QQS as a component of the starch metabolic network in Arabidopsis leaves. *Plant J.* 58(3):485-498.

Manpreet S. Katari

NYU Center for Genomics and Systems Biology, New York University, New York, USA

Manpreet S. Katari is a Clinical Assistant Professor in the Department of Biology at NYU, who leads bioinformatic research projects at the Center for Genomics and Systems Biology. He received his PhD in Genetics from Stony Brook University in New York and performed his PhD research in Comparative Genomics at Cold Spring Harbor Genome Center under the supervision of Dr. W.R. McCombie. For his NIH postdoctoral fellowship, Dr. Katari joined NYU's Center for Genomics and Systems Biology. In Dr. Gloria Coruzzi's Plant Systems Biology group, Dr. Katari was the lead developer for VirtualPlant, a software that enables biologists to analyze their –omic data in a Systems Biology context. Dr. Katari's research interests lie in the areas of data integration and translational systems biology research. He has developed courses and workshops to train students and researchers in developing bioinformatics skills in analysis of genomic data. Highlights include: "Bioinformatics in Medicine and Biology" and "Biological Databases and Data Mining". In addition to these formal courses taught at NYU, Dr. Katari leads "R-boot Camp" workshops attended by students, scientists and PIs at NYU and internationally.

Abstract

VirtualPlant: A software platform for translational systems biology research in crops

To enable Systems Biology studies across plant species, we have developed and expanded the VirtualPlant software platform to include Maize, Rice and Arabidopsis genomes. VirtualPlant (www.virtualplant.org), originally developed for an NSF Arabidopsis 2010 Grant, includes tools for data analysis, integration and visualization such as the Arabidopsis MultiNetwork interaction database, as well as novel data visualization tools including BioMaps and Sungear [1,2]. VirtualPlant enables seamless integration of data and tools into a single software platform by virtue of its unique "Gene Cart", which enables researchers to store results, enabling iterative cycles of analysis, a highlight of Systems Biology [3]. Additionally, VirtualPlant's web-based user-friendly GUI enables plant biologists to analyze their own genomic data, enabling them to uncover and infer biological insights.

VirtualPlant has played an integral part in enabling Plant Systems Biology research in Arabidopsis in prominent plant labs around the world. Our recent expansion of VirtualPlant to include important crops such as Maize and Rice, facilitates the comparisons of crop networks to Arabidopsis, hence enabling translational research. For example, VirtualPlant allows researchers to predict how an interacting network of genes/products in crop genomes will react as a system in response to an environmental change or genetic modifications, and predict genes in the crop to target for manipulation. Here, we present a case study of how VirtualPlant Rice can be used to enable hypothesis generation regarding Nitrogen-responsive gene networks in Rice, by transferring "network knowledge" of gene interactions (e.g. protein-protein, protein-DNA, etc) from Arabidopsis. Currently, VirtualPlant is cross-operational across Arabidopsis, Maize and Rice enabling translational network analysis. Additional crop genomes including Soy, Brassica, Medicago, Grape, Tomato, Sorghum, and Poplar will be inducted into VirtualPlant in the near future.

[1] Katari, MS, Nowicki, SD, Aceituno, FF, Nero, D, Kelfer, J, Thompson, LP, Cabello, JM, Davidson, RS, Goldberg, AP, Shasha, DE, Coruzzi, GM, and Gutierrez, RA, VirtualPlant: a software platform to support systems biology research. *Plant Physiol,* 2010. 152(2): p. 500-515.

[2] Poultney, CS, Gutierrez, RA, Katari, MS, Gifford, ML, Paley, WB, Coruzzi, GM, and Shasha, DE, Sungear: interactive visualization and functional analysis of genomic datasets. *Bioinformatics*, 2007. 23(2): p. 259-261.

[3] Gutierrez, RA, Shasha, DE, and Coruzzi, GM, Systems biology for the virtual plant. *Plant Physiol*, 2005. 138(2): p. 550-554.

Anne Ingeborg Myhr

GenØk – Centre for Biosafety, Forskningsparken, Tromsø, Norway

Anne Ingeborg Myhr is Acting Director at GenØk - Centre for Biosafety. She holds a Master's degree in Biotechnology from NTNU, Trondheim, and a PhD from the Institute of Medical Biology at the University of Tromsø. Her main experience is within risk assessment and risk management of GMOs as well as on ethical, social and legal aspects (ELSA) related to genetic engineering and nanobiotechnology.

Abstract

Sustainability and ethical perspectives in innovation processes within agriculture with a special focus on GMOs

There is an increasing interest in identification of preconditions for introduction of new emerging biotechnologies within agriculture. New technologies are characterized by uncertainty with respect to the expected beneficial effects as well as possible adverse effects. They may have a major impact on social development, raising questions about whether the development is desirable, for whom, as well as the impact decisions made now may have on future options. One of the main challenges with new technologies is how to use the new opportunities these technologies offer us, while at the same time considering how we take care of biodiversity, promote sustainability and prevent harmful effects to health. This has caused an increased interest in that ethical, legal and social perspectives are included in the research process itself, as well as on the technological applications developed through it, to ensure that it can be socially robust, ethically sound, economically viable and environmentally responsible. The importance of including such perspectives in the process of research and development may also help identify and priorities research needs, and it may support evaluations of the adequacy of relevant existing regulatory frameworks. These challenges will be described in this presentation together with a discussion of implications by including concepts as sustainability in relation to introduction and use of new technologies as GMOs within agriculture.

André van Steirteghem

Secretary of Committee on Publication Ethics (COPE) and Professor-emeritus Vrije Universiteit Brussel, Belgium

André Van Steirteghem graduated medicine at the VUB, where where he also trained in paediatrics and clinical pathology. He worked in a medical mission in Central Africa in 1970 and was a Visiting Scientist at the Clinical Centre of the National Institutes of Health (Bethesda, Maryland, USA) from 1974 to 1977). In 1979, he obtained his PhD at the VUB. Following his return to Belgium he became Laboratory and Scientific Director of the VUB Centre for Reproductive Medicine until 2005, when he became Emeritus Professor. He had several responsibilities at the VUB: Chairman of the Medical Board, Vice Rector of the University and Dean of the Medical School. For four years he was a member of the Belgian National Committee of Bio-Ethics and he is currently chairman of the Belgian Federal Commission for the Protection of the Human Embryo in Vitro. He is a member of the Belgian Royal Academy of Medicine. He has been an author, Associate Editor, Deputy Editor (2005–06) and Editor-in-Chief (2007 to present) for *Human Reproduction*. His extensive publication list covers most developments in the area of reproductive medicine and science of the past three decades.

Abstract

Research and Publication Ethics

Research results in a publication and therefore publication ethics is an intrinsic part of research ethics. The lecture will answer the question whether there is a reason for concern by examining the evolution of retractions in the literature. Not just high profile cases of Falsification, Fabrication or Plagiarism (the big FFP) are a concern but there is the magnitude of QRP (Questionable Research Practices). What can The Committee on Publication Ethics (COPE) do to promote ethical attitudes regarding research ethics? Key players in prevention of or dealing with research misconduct are the universities & research institutes, the funding agencies, the academies and journals & publishers.

COPE website: http://publicationethics.org/

Anna Amtmann

University of Glasgow, College of Medical, Veterinary and Life Sciences, Glasgow, Scotland, UK

Anna Amtmann obtained her PhD from the University of Göttingen in Germany and subsequently worked as post-doctoral fellow in the laboratory of Dale Sanders at the University of York, UK, before taking up a Lecturer position at the University of Glasgow, UK, in 2001. She is an internationally recognized expert in the molecular mechanisms that enable plants to adapt to mineral nutrient deficiency, salinity and drought. Her research is holistic, addressing questions relating to ion transport, metabolism, transcriptional regulation and epigenetics through cutting-edge technologies such as patch clamping, confocal microscopy, microarrays, and next-generation sequencing. While *Arabidopsis thaliana* and related wild halophytes are preferred experimental systems in her laboratory, fundamental findings from these model plants are being translated into crops in collaboration with the James Hutton Institute, the John Innes Centre and companies, e.g. Bayer CropScience. Current research projects investigate opportunities to improve plant growth and drought tolerance through epigenetic engineering (BBSRC funded), to increase pest resistance in oilseed rape (BBSRC funded), to use synthetic biology to desalinate water (EPSRC funded) and to discover the genes that adjust root architecture to mineral nutrient supply (Gatsby Trust funded). Anna Amtmann was elected chair of the Gordon Research Conference on Salt and Water Stress in Plants in 2010. She is Associate Editor for Plant Phsyiology and Plant, Cell & Environment, and she is a member of the steering committee of the BBSRC Crop Improvement Research Club (CIRC).

Abstract

The role of histone modifications for somatic stress memory in plants

In arid and semi-arid environments, drought and soil salinity usually occur at the beginning and end of a plant's life cycle, offering a natural opportunity for the priming of young plants to enhance stress tolerance in mature plants. Chromatin marks, such as histone modifications, provide a potential molecular mechanism for priming plants to environmental stresses, but whether transient exposure of seedlings to hyperosmotic stress leads to chromatin changes that are maintained throughout vegetative growth remains unclear. We have established an effective protocol for hyperosmotic priming in the model plant *Arabidopsis,* which includes a transient mild salt treatment of seedlings followed by an extensive period of growth in control conditions. Primed plants are identical to non-primed plants in growth and development, yet they display reduced salt uptake and enhanced drought tolerance after a second stress exposure. ChIP-seq analysis of four histone modifications revealed that the priming treatment altered the epigenomic landscape; the changes were small but they were specific for the treated tissue, varied in number and direction depending on the modification, and preferentially targeted transcription factors. Notably, priming leads to shortening and fractionation of H3K27me3 islands. This effect fades over time, but is still apparent after a ten day growth period in control conditions. Several genes with priming-induced differences in H3K27me3 showed altered transcriptional responsiveness to the second stress treatment. We conclude that the experience of transient hyperosmotic stress by young plants is stored in a long-term somatic memory comprising differences of chromatin status, transcriptional responsiveness and whole plant physiology.

Sani E, Herzyk P, Perrella G, Colot V, Amtmann A (2013) Hyperosmotic priming of *Arabidopsis* seedlings establishes a long-term somatic memory accompanied by specific changes of the epigenome. *Genome Biology* (in press)

Ortrun Mittelsten Scheid

Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna, Austria

Ortrun Mittelsten Scheid is currently group leader studying epigenetics (or non-Mendelian genetics) at the Gregor Mendel Institute (GMI) in Vienna, Austria. Much more fascinated by zoology than botany during her studies in Germany, she discovered the extraordinary features of plants as experimental organisms late, but not too late. Coming to Switzerland with a two-year stipend resulted in many years more, first at the ETH Zürich with Ingo Potrykus and then at the Friedrich Miescher Institute in Basel with Jerzy Paszkowski. Her postdoc time was so inspiring and rewarding that she did not want to move on. However, Ortrun Mittelsten Scheid decided to move to Vienna in Austria where a new plant research institute had been founded. When she arrived, there was only in a hole in the ground, but she did not regret the decision: now the GMI is a wonderful place to work, with great people and fantastic infrastructure.

Abstract

Inheritance of acquired traits: a debate with a long history

Inheritance, the recurrence of characteristics between generations, was noticed already early in human history. It was widely studied because of its fundamental role in domestication and breeding of plants and animals, and there were numerous speculations about how traits are maintained or changed from parents to progeny. However, only the systematic and controlled experiments of Gregor Mendel in the 19th century (with plants as experimental models, by the way) prepared the ground to understand the underlying principles. After a lag phase, the new science, termed genetics, became one of the dominating and most fruitful research fields of the 20th century. With the proof of DNA as the determinant of traits, the discovery of the double-helical structure and the genetic code, and the compilation of complete genome sequences, DNA as the sole carrier of heritable information was nearly invariably accepted. So was the similarly important breakthrough of the 19th century: the paradigm of Darwinian evolution. Its prerequisite of random variation of traits was, and still is, in good agreement with the randomness of most changes in the DNA sequence. Modern concepts in genetics and evolution seemed to have excluded historic ideas about directed or acquired changes of heritable traits if they were not DNA-encoded. However, there is now substantial research activity summarized as epigenetics, to understand the role of chromatin, RNA, and nuclear organization for heritable states of gene expression and resulting traits of cells, tissues, and organisms. In the light of current results, inheritance in addition to the DNA sequence, including environmentally induced changes, appears conceptually possible. Numerous studies describe already “transgenerational inheritance” of exogenous influence. Is this a recurrence of presumably dead concepts?

Louwrance Peter Wright

Department of Biochemistry, Max Planck Institute for Chemical Ecology, Jena, Germany

Louwrance Wright was born and educated in South Africa where he obtained his Ph.D. in Biochemistry at the University of Pretoria. His Ph.D. focussed on the effect of tea (*Camellia sinensis*) polyphenols on the quality of black tea produced in Central and Southern Africa. After his Ph.D. he did a short stretch of contract research for the Tea Research Foundation of Central Africa. He obtained a post-doctoral position at the Forestry and Agricultural Biotechnology Institute (FABI) in Pretoria, where he investigated the population genetics of the pathogenic fungi *Cylindrocadium parasiticum* and *C. pauciramosum*, and monitored disease outbreaks in commercial forests in Africa. Louwrance Wright is currently a principal investigator at the Max Planck Institute for Chemical Ecology, studying the regulation of the methylerythritol phosphate pathway of terpenoid biosynthesis by using the approach of Metabolic Control Analysis.

Abstract

Isotopic labeling of the MEP pathway in *Arabidopsis thaliana* lines over-expressing 1-deoxyxylylose phosphate synthase, shows the existence of a second methylerythritol cyclodiphosphate metabolite pool

The chloroplast localized methyl erythritol phosphate (MEP) pathway produces the precursors of photosynthetic pigments, membrane linkage groups of photosynthesis and redox components, and many other isoprenoid-derived hormones and natural products. In this pathway, the central metabolic intermediates pyruvate and glyceraldehyde-3-phosphate are diverted towards isoprenoid biosynthesis by 1-deoxyxylulose 5-phosphate synthase (DXS). We examined the control exerted by DXS on the flux in this pathway using metabolic control analysis and a suite of transgenic, RNAi, and natural mutant lines with a range of DXS activities. Direct kinetic data were obtained from a $^{13}CO_2$ labeling system where *Arabidopsis thaliana* was labeled under natural growth conditions in a dynamic flow, climate controlled, gas exchange cuvette. HPLC-MS/MS analysis of ^{13}C incorporation into DXP in plant extracts coupled to measurements of DXS activity in the same leaf material showed that DXS has a high flux control coefficient (0.82) under photosynthetic steady state conditions. The maximum amount of ^{13}C incorporation into DXP for all lines under these conditions was about 60%. Upon examining the downstream intermediate methylerythritol cyclodiphosphate (MEcPP), we noted a four-fold increase in MEcPP concentration for all plant lines constitutively over-expressing DXS and a noticeable drop in the maximum ^{13}C incorporation from 60% in the wild type to 30%. However, maximum labeling of one of the pathway's end products dimethylallyl diphosphate (DMAPP) only reached 30% in all lines. We conclude that up-regulation of DXS triggers a natural efflux mechanism causing MEcPP to be diverted out of the MEP pathway. This second pool of MEcPP is responsible for the decrease of 13C incorporation into total MEcPP. Thus, disruption of the MEP pathway flux results in a substantial diversion of flux into a second, physically isolated pool of MEcPP, possibly located in the cytosol. Consequently only a limited amount of the increased flux resulting from DXS up-regulation reached downstream end products such as chlorophylls and carotenoids.

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Uwe Sauer earned his MS and PhD in microbiology from the University of Göttingen. During his postdoc work on metabolic engineering in the chemical engineering lab of Jay Bailey in Zurich, he became interested in computational modelling of cellular behaviour and quantitative analysis of intracellular fluxes in particular. In the late nineties, his research began to focus on quantitative understanding of interactions and regulation within complex microbial metabolic networks by using and developing cutting-edge experimental and computational methods. For this purpose, research in the Sauer lab takes an interdisciplinary approach that combines quantitative experimentation and modelling to solve fundamental questions of complex metabolic network operation, allowing to engineer general cellular functions such as redox and energy metabolism. In particular, his lab has pioneered methods for 13C-flux analysis and is a key player in quantitative metabolomics, such that he became a well-recognized expert on central metabolism of bacteria and yeast. Uwe Sauer has over 70 publications in peer-reviewed journals over the last 5 years, and is a member of various international editorial boards, scientific steering and advisory committees of international organisations and companies in systems biology and biotechnology.

Abstract

How do microbes coordinate their fluxes

While our knowledge on regulation events is steadily increasing, we are much less informed about the functionality of individual regulation events and their quantitative relevance for controlling a given biological function. For metabolism this function is the flux of small molecules that can be quantified network-wide through methods of ^{13}C-flux analysis [1]. This ability to quantify metabolic function allows us to investigate the question which of the multiple overlapping regulation mechanisms are employed by microbial cells to manage their small molecule traffic [2, 3]. By combining various omics methods with computational analysis, we delineate actively controlling regulation events from the much larger number of co-occurring regulation events. From large-scale ^{13}C-flux experiments with deletion mutants in different microbes we found, somewhat surprisingly, that only few transcription factors are actively involved in controlling the distribution of flux in the network. Here I will discuss the relevance of enzyme phosphorylation and allosteric metabolite-enzyme interactions in controlling central metabolic fluxes.

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Patrick van Dijck obtained his PhD degree in 1991 on mechanisms of transcriptional activation of androgen and estrogen regulated genes. A first postdoc was performed in the Laboratory of Molecular Cell Biology at the KU Leuven on trehalose metabolism and yeast stress resistance mechanisms. After a second postdoc at Janssen Pharmaceutica (J&J) between 1995 and 1997, he returned to the KU Leuven to become a group leader on a VIB-sponsored project. Since 2002 he is group leader of the VIB department of Molecular Microbiology and since 2003 professor at the KU Leuven. He is investigating the role of plant trehalose metabolism and he studies nutrient-induced signal transduction pathways that affect morphogenesis and virulence in the human fungal pathogen Candida albicans. He has strong expertise in biofilm work and antifungal drug resistance mechanisms. Patrick published 100 refereed manuscripts and has 15 patent applications of which several are granted and licensed. He has extensive experience in managing and coordinating research projects, both in Flanders as well as abroad. He was coordinator of a MC ITN training network.

Abstract

Investigation into the function of trehalose metabolism enzymes in higher plants

Trehalose functions as a reserve carbohydrate and a stress protectant in a large variety of microorganisms, insects and invertebrates. The most widely distributed pathway to synthesize trehalose in nature consists of two consecutive enzymatic reactions with a trehalose-6-P (T6P)-synthase (TPS) enzyme, producing the intermediate T6P, and a T6P-phosphatase (TPP) enzyme, which dephosphorylates T6P to produce trehalose and inorganic phosphate. In plants these enzymes are called Class I and Class II proteins respectively. In *Arabidopsis thaliana* there are four Class I genes and 7 class II genes and from these 11 genes only one shows synthase activity (AtTPS1). The Class II proteins possess both TPS and TPP consensus regions, but appear to have lost enzymatic activity during evolution. This loss seems to have been complemented by an extra group of enzymes (10 in *A. thaliana*) of small protein size with little sequence homology to the Class I and Class II proteins, but which all are active TPPs. Phylogenetic analysis between different plant species and microbial species shows that the three groups of enzymes are already present in the most primitive plants and that they originate from bacteria. Expression analysis using promoter-GUS-GFP lines for all 21 Arabidopsis genes shows a clear cell and/or organ specific and condition specific expression pattern. To start the investigation on the role of specific genes, we focused first on the TPP enzymes. Analysis of a few TPP OX and KO lines shows that these proteins and/or their catalytic activity play an important role in plant development and in crosstalk to plant growth regulators, such as auxin and abscisic acid.

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Junji Yamaguchi obtained his PhD from Nagoya University in 1986. After postdoctoral fellow of Chua's Lab at Rockefeller University 1986-1987, he got a job at the Nagoya University (Assistant and Associate Professor 1987-2000). In 2001, he moved to Sapporo, Hokkaido, northern island of Japan. His recent research is focused on two areas, the function of ubiquitin proteasome system in plants, and on mechanisms related to plant immunity. They mainly use the convenient laboratory plant Arabidopsis, which allows the parallel use of classical and molecular genetics. Junji Yamaguchi and his colleaques are also interested in studying on protein-protein interactions by yeast two-hybrid and/or immuno-coprecipitation methods as well as through proteomics approaches.

Abstract

ATL31 ubiquitin ligase, a regulator of the C/N response in Arabidopsis, is also involved in defense response mediated by membrane traffic system

In higher plants, the metabolism of carbohydrate (C) and nitrogen nutrients (N) is mutually regulated and referred to as the C and N balance (C/N). Plants are thus able to optimize their growth depending on their cellular C/N status. Arabidopsis ATL31 and ATL6 encode a RING-type ubiquitin ligase and play a critical role in C/N regulation [1]. A controlled level of ATL31 activity is essential to ensure growth correctly arrests when C/N conditions are unfavourable and to prevent aberrant arrest under suitable conditions. Proteome analysis revealed that 14-3-3 proteins are associated with ATL31, and the interaction between ATL31 and 14-3-3χ is confirmed by yeast two-hybrid and co-immunoprecipitation analyses. *In vitro* assays showed that 14-3-3χ can act as a target of ATL31, which catalysed ubiquitination of the recombinant protein. Degradation of 14-3-3χ *in vivo* was also shown to be correlated with ATL31 activity, and to occur in a proteasome-dependent manner. Furthermore, a reduction in 14-3-3 protein accumulation was induced by a typical shift to high C/N conditions in Arabidopsis seedlings, which was dependent on the presence of ATL31 and ATL6. These results indicate that ATL31 functions to target and ubiquitinate 14-3-3 proteins for degradation via the ubiquitin-proteasome system during the response to cellular C/N status [2].
The C/N has an important role not only in the growth and development but also in the defense response. However, the mechanism to connect C/N regulation and defense response has been poorly understood. We demonstrated that C/N regulator ATL31 has an important role in resistance against *Pseudomonas syringae* pv. *tomato* DC3000 [3]. Proteomic analysis and micro array database search identified plasma membrane-localized SNARE SYP121 as novel ATL31 interactors. Since SYP121 has been reported as the essential factor for plant penetration resistance against to powdery mildew, we have verified whether the ATL31 has function in penetration resistance. Microscopic analysis demonstrated that ATL31 is specifically localized at fungal penetration site as well as SYP121. *ATL31* overexpressor revealed early induction in papilla formation and increased penetration resistance. Furthermore, *syp121-1* showed hypersensitivity to C/N stress conditions. These results indicate that the ATL31 positively controls plant penetration resistance with the SYP121 which is mediated by membrane traffic system.

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Matin Qaim is Professor of International Food Economics and Rural Development at the University of Göttingen, Germany. He holds a PhD in agricultural economics and has extensive research experience related to food security and potentials for productivity increases in the small farm sector of developing countries. He has carried out research projects in various countries of Africa, Asia, and Latin America, including studies on the role of genetically modified crops. Matin Qaim has published widely in high-ranking journals and has been awarded different academic prizes. He is member of several scientific and policy advisory committees.

Abstract

Genetically modified crops, food security, and sustainable agriculture

The role of genetically modified (GM) crops to contribute to food security and sustainable development is the subject of public controversy. Often, public attitudes are not formed on the basis of the best scientific information. In this talk, possible pathways of how GM crops could contribute to improved food security are discussed and an overview of the evidence so far is provided. The talk will especially focus on what is known about socioeconomic and environmental impacts of GM crops in the context of developing countries, with examples from herbicide-tolerant soybeans in South America and insect-resistant cotton in Asia. Widespread public concerns will also be scrutinized in this connection. One conclusion is that GM crops are not a panacea and should not be considered as a substitute for other technical and institutional innovations. Yet, the evidence suggests that they can contribute importantly towards food security and sustainable development. These potentials of GM crops are underrated in the public debate, while risks and concerns are often overblown. Better science communication is needed.

Notes

Poster Abstracts

P 01| The plant cytoskeleton as a complex network: dynamics of actin filaments and cytoskeleton efficiency

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The plant cytoskeleton is a highly dynamical network comprising actin filaments and microtubules. While the molecular mechanisms of its formation and maintenance are intensively investigated [1], little is known about the organizational principles that shape these processes on the whole-cell level. Here, we develop a framework that allows the quantification and analysis of the cytoskeleton as a complex network. First, we test the method by applying it to the phenomenon of microtubule reorientation in dark grown, expanding plant cells when exposed to light and confirm and extend the results of previous studies [2]. Second, we quantify the impact of light also on the actin network. While mircotubules orient vertically under light, the actin aligns more horizontally and its distribution in the cell becomes more heterogeneous. Third, we demonstrate that the networks of both actin filaments and microtubules display biologically desirable properties such as short path lengths and high robustness. These properties are kept constant over time. In support of studies on actin and microtubule array formation [3], further time series analyses of the network properties reveal ("causal") interdependence of the two cytoskeletal components.

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P 02| Cell walls and cotton fibre development

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The developing cotton fibre is an excellent model to study cell wall biochemistry, structure and function as it is a single cell with an unusual capacity for cell elongation and cell wall synthesis. Mature cotton fibres are comprised mostly of cellulose, nonetheless the primary cell wall of developing fibres contains non-cellulosic polysaccharides and glycoproteins that function in its growth and development and, ultimately, may determine quality traits such as fibre length, fineness and mechanical properties. Using monoclonal antibodies, a range of developmental dynamics of several non-cellulosic polysaccharide epitopes have been identified in developing cotton fibres. For example, the esterified homogalacturonan (LM20) epitope is not detected after fibre elongation as a more rigid cell wall is formed. Detection of the galactan (LM5) epitope is lost earlier during development compared to the arabinan (LM6) epitope suggesting developmental changes in the RG-I structure at early stages of fibre development. Moreover, two novel cellular structures were identified in the developing cotton fibre. The first structure, named as polysaccharide-rich intercellular spaces (PRIS), contained sets of particles to which the LM15 (xyloglucan) and LM19 (non-esterified homogalacturonan) probes bound. PRIS were mainly found in the developing fibre tissue during the elongation stage (from 9 to 17 days post anthesis) suggesting a possible role in fibre cell adhesion. A second structure consisted of a distinctive pattern of paired cell wall bulges between neighbouring cell walls, which contained xyloglucan, and are present through the elongation stage into the early maturation stage (22 days post anthesis).

P 03| Epitope Detection Chromatography; an insight into the complexity of plant cell wall

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The structure and interactions of cell wall polysaccharides is poorly understood, especially with regards to the mechanical adaptations and dynamics of the cell wall. Studying the composition of cell walls from different plant species, cell types or at different stages of development might be the key to elucidate the biological functions of polysaccharides. However, the current methods of study, usually based on chemical and physical analysis, involve large amounts of plant material making it impossible to refine the analysis to a specific tissue or cell type. In the last twenty years, a better understanding of the variation in cell wall architecture has been facilitated by the use of immunolabelling with large collections of monoclonal antibodies.
However, this technique has its limitation with the possible occurrence of epitope masking. Indeed the polysaccharide matrix can be so dense that some features or epitopes would be hidden, not allowing a specific monoclonal antibody to access its epitope. Here we present a new technique called Epitope Detection Chromatography (EDC). This method uses the resolution power of chromatography combined with the high sensitivity of monoclonal antibodies and immunoassays. The EDC's exquisite sensitivity allows working on micro-scale samples (tissue, cell type, organs) but, as the polysaccharides are extracted, is not subject to masking as immunolabelling can be. Here we present progress made in developing the EDC method.

P 04| Structure of Pectin in Citrus Fruits and Functionality

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The structure of pectin is based on a backbone of polygalacturonic acid, which is called homogalacturonan region- HG [1]. Besides, segments consisting of alternating sequences of L-rhamnosyl and D-galacturonosyl residues, ramified with side chains of arabinans, arabinogalactans and galactans, are called Rhamnogalacturonan I - RGI [2]. Commercial pectin is mainly derived from citrus peels. Pectin exhibit functional properties like gelling, thickening, and emulsifying that are widely used in food industry. The project aims to find out the variation of pectin components depending on plant sources and processing in relation with functional properties.
In this study, dried citrus fruit peels (orange, grapefruit, lime and lemon) were used as raw material for pectin extraction. The peels were subjected to HCl, HNO_3 and $H_2C_2O_4$ extraction conditions. The pHs were 1.5, 2.1, and 4.6 respectively. Pectins were obtained on laboratory scale (HCl and $H_2C_2O_4$) and industrial scale (HNO_3).
The extraction conditions affected intrinsic viscosity and molecular weight of pectins significantly. In regard to monosaccharide composition, GalA/ rhamnose ratio (mol %) of pectins extracted by oxalic acid was higher than the others. Additionally, the degree of branching (Ara + Gal/ Rha mol %) was higher in pectins extracted by hydrochloric acid and oxalic acid. Alterations in the composition and structure of the pectins due to the conditions of extraction will be reported.

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P 05| A tool for phenotyping based on mobile terminals – technical details

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We are developing a phenotyping system for scientists and breeders that can be implemented without major investments. The system uses personal digital assistants (PDA) to enter phenotyping data on-site into user defined forms, so called 'phenotyping schemes'.
For the software development, we are focusing on free (and open source) software: Apache Struts 2 and Apache Tomcat 6 (web page for defining phenotyping schemes), Apache httpd2 web server (web page for distribution), MySQL 5 (underlying database). Extensible markup language (XML) is used for exchanging data between database and PDA. Well-established free software for the work with PDAs is still unavailable. Thus, a commercial programming platform from Microsoft™ with an integrated development environment (Visual Studio 2008) was used for the development of the user interface for the PDAs.
A main limitation of PDAs is the small screen size which makes it difficult to get an overview over larger datasets, e.g. field plans. Therefore, we are testing the use of heavy-duty tablet PCs for data collection. With the use of larger displays we develop tools to review current data and compare them with older data on-site. Furthermore, we use the larger screen to display images to illustrate plant phenotypes. This will facilitate a uniform and reproducible phenotyping not only by less experienced staff but also within projects involving separate groups at different locations.

P 06| Yield penalty and predictability of drought tolerance in potato

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Global change models for Central Europe predict increased likelihood of early summer drought, which will reduce yield especially in summer crops like potato (*Solanum tuberosum*). We studied the drought stress response of potato cultivars to gain insight into genetic variance, predictability of field performance and potential costs of tolerance. Tuber yield and starch content were determined for 34 potato cultivars cultivated at optimal and reduced water supply in greenhouse trials with pot-grown plants and field experiments.
Based on data from the experiments, we generated an artificial data set to tested several stress indices for their power to distinguish tolerant and sensitive genotypes independent of the yield potential. We identified the indices SSI (stress susceptibility index) and DSY (deviation of relative starch yield from its experimental median) to be the most efficient indices.
The European potato cultivar population contains a small but significant genetic variance for drought tolerance as a basis for breeding and the search for tolerance markers. Under field conditions, we found a negative correlation between drought tolerance and yield potential, suggesting that there might be a yield penalty for increased drought tolerance. The size of the yield penalty for drought tolerance, the frequency of yield-decreasing drought and the costs for the identification of tolerant germplasm affect the economic feasibility of tolerance breeding.

P 07| Phosphoinositides regulate cell wall deposition in Arabidopsis leaves

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Plant cell-wall assembly involves a complex pathway were different macromolecules are deposited into the cell wall to form an intricate matrix. Cellulose is one of the most abundant components of plant cell walls. It forms a part of the load-bearing network that both controls and maintains cell shape, while allowing regulated cell expansion that is essential during growth. The cellulose microfibrils are deposited by cellulose synthase (CESA) complexes, which are secreted to the plasma membrane by Golgi derived vesicles. The recycling of the CESAs is likely executed by a tight interplay of exocytosis and endocytosis, which in turn is controlled by various factors.
The $PI(4,5)P_2$ is one class of membrane lipids which has emerged as a key component in regulating membrane trafficking [1]. These bi-phosphates are one of the major by-products of the plant phosphoinositide (PI) pathway, catalyzed by specific PIP-Kinases (PIPKs) by phosphorylation at the 5^{th} position of the inositol ring. Our results show that a double *pipk* mutant, affecting *PIP5K1* and *PIP5K2*, cause a six-fold increase in epidermal cell wall thickness in leaves, and almost a three-fold increase in the cellulose levels. Various other cell wall components were also altered in these double mutants. Fluorescently tagged versions of these kinases localize to distinct but dynamic foci at the plasma membrane, which strongly suggests a role for them in endocytosis. We will investigate the exact mechanisms of how these phosphoinositides are involved in regulating membrane trafficking events by using various molecular biology techniques and advanced imaging tools.

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P 08| Peptide Transport – Identification of important amino acids in AtPTR1 by forward genetics

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Transport of small peptides of two to three amino acids is suggested to be important for translocation of organic nitrogen within the plant, plant nutrition, flowering and seed development as well as for senescence. The plasma membrane-localized peptide transporter PTR1 of *Arabidopsis thaliana* contains 12 predicted transmembrane domains and mediates transport of di- and tripeptides with low selectivity and high affinity (1, 2). Consistent with AtPTR1 transport properties and the expression in Arabidopsis roots, T-DNA insertion lines display reduced growth compared to wild type plants on medium with dipeptides as nitrogen source, while overexpressing lines (35S::AtPTR5) produce more biomass (3).
Five lines with a loss-of-function (*ptr1*-like) phenotype were identified by a forward genetic approach. Three lines had mutations in *AtPTR1*, (i) leading to a premature stop, (ii) causing an amino acid exchange G502E in TMD11, or (iii) carrying two mutations i.e. resulting in a G85E exchange in TMD2 and a replacement of G524S in the region between TMD11 and TMD12. The role of these amino acids in dipeptide transport, regulation or targeting is currently being investigated.
Furthermore, two lines with mutations in genes other than *AtPTR1* were isolated and next generation sequencing is in progress to identify the mutated loci.

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P 09| Functional Specialization of *Arabidopsis* Poly(A) Polymerases

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My PhD research aims to shed light on the differential functions of plant poly(A) polymerases (PAPs). The *Arabidopsis thaliana* genome encodes three nuclear PAPs. All three PAPs are expressed in all plant tissues at all developmental stages. While knocking down one particular PAP leads to specific growth defects in the plant and an early flowering phenotype, knocking out the other two PAPs causes a delay in flowering time. This implies a functional specialization of the different PAPs despite their general enzymatic function of polyadenylating mRNAs and demonstrates a connection of poly(A) polymerases to the regulatory network of flowering time control.
By crossing *pap* mutants to flowering-time mutants, I have begun to unravel the functions of poly(A) polymerases in these networks. To reveal the molecular processes underlying the *pap* mutant phenotype, poly(A) tail lengths and expression levels of RNAs involved in the flowering time control are determined. Strikingly, the floral repressor FLC, which is regulated by its own antisense RNA [1] is differentially expressed in *pap* mutants.
An analysis of the transcriptome of seedlings and flowers by RNA-Seq revealed profound changes at the transcriptional level in PAP mutants. Defining the differential functions of canonical PAPs could reveal a novel general regulatory mechanism in gene expression that might also be detected in other organism groups with more than one canonical PAP.

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P 10| The dynamics of phosphorylation signaling events in *Arabidopsis thaliana* - network inference from time-resolved phospho-proteomics data

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Elucidating the dynamics of molecular processes in living organisms in response to external perturbations is one of the central goals of systems biology. We investigated the dynamics of protein phosphorylation events in *Arabidopsis thaliana* (Ath) exposed to six nutrient starvation conditions and subsequent resupply and one control condition. Phosphopeptide levels were measured at five consecutive time points at 0, 3, 5, 10, and 30min after nutrient resupply, respectively. When embedded in the protein-protein interaction network (PIN) of Ath, phosphoproteins were identified to be associated with a higher PIN degree compared to average proteins. Based on the obtained time-series data, we reconstructed the molecular interaction network. We assessed the performance of different network inference methods relative to the successful prediction of intra-organellar interactions as annotated in the SUBA and AtPIN databases. Graphical Gaussian models proved to work best. The topology of the inferred networks corresponded to an information dissemination architecture with the average in-degree being smaller than the out-degree. Hub proteins were found to be associated with kinase and transporter functions. Our results demonstrate that modern proteomics technologies allow monitoring time-resolved phosphorylation cascade events and, combined with established network inference methods, novel insight into the molecular signaling events following external perturbations can be obtained.

P 11| *In Vivo* Stable Isotope Tracing of Interactions of Leaf Development

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Measuring the metabolite profile of plants can be a strong phenotyping tool but often the changes of metabolite pool sizes are difficult to interpret, not least because metabolite pool sizes may stay constant while the fluxes are altered and vice versa. Hence, following the carbon of metabolites enables a better understanding of the metabolic phenotype.
We developed an *in vivo* plant feeding method were labelled precursors are loaded into the plant using a reverse petiole assay [1]. This assay was applied to a single leaf analysis of Arabidopsis rosettes and integrated into a metabolite profiling analysis.
This assay is suitable for plants grown on soil in a phytotron, but could equally be applied to plants growing in a greenhouse or even in the field.

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P 12| Plant-produced therapeutical vaccine for chronic Hepatitis B carriers

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The Hepatitis B Virus (HBV) causes one of the most prevalent human diseases. Acute or chronic hepatitis B lead to liver dysfunction, cirrhosis or hepatocellular carcinoma. Despite the fact that preventive anti-HBV vaccines have been available for almost 30 years, the number of chronic carriers is permanently growing, mainly in developing countries.
HBV is an enveloped virus with a double-stranded DNA genome. The envelope of HBV consists of lipid membrane with three surface proteins S, M and L. The S protein contains 226 amino acids, the M protein has an additional domain preS2 (55 aa), the L protein carries preS2 and preS1 domain (108 or 119 aa). Both regions may play an important role as vaccine components. Several lines of evidence lead to conclusion, that the inclusion of preS domains in a vaccine intensify its efficacy. Another component of a possible therapeutical vaccine is Hepatitis B core protein (HBcAg). That antigen due to its capability to self-assemble into a form of durable Capsid-Like Particles (CLPs) is an unusually strong immunogen. HBcAg antigen dimer forms a structure like an upside down „T" letter. The most exposed substructure is a small loop on the tip of the spike, which forms the major epitope of HBcAg. The fact that major epitope is displayed on the particle surface affects on exceedingly high immunogenicity of HBcAg. Furthermore those properties can be transferred to foreign epitopes, including preS domains.
At present, there are several reports on the successful expression of HBcAg particles in transgenic plants, especially in transient expression. Above mentioned features of HBcAg make the HBV core protein a promising candidate for becoming an effective component of therapeutical vaccine against chronic hepatitis B

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P 13| Systemic RNA Transport into Reproductive Tissues

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Numerous mRNAs and non-coding RNAs are transferred via plasmodesmata into and via the phloem tissue to distant organs. To test RNA transport and potential transport motifs we expressed dominant negative acting RNA. The presence of the artificial RNA molecules interfering with DMC1 a key factor for meiotic progression results in sterile plants. We constructed siRNA and miRNA molecules interfering with *DMC1* transcripts, and dominant negative acting *DMC1* mRNA fusion sequences interfering with protein activity to evaluate the pace and extent of RNA transport to distant organs. To test RNA mobility we locally expressed the RNAs in source tissues of transgenic plants (stocks) grafted with wild-type plants (scions). Transport and interference with DMC1 function in reproductive wild-type organs results in abnormal meiosis and in sterility or abnormal shaped pollen. After grafting and induction of expression in transgenic stock plants wild-type flowers formed on scions which produced abnormal pollen or were sterile. Thus, the RNA molecules were moving over long distances from source leaves to flower organs. Within flowers the allocated small RNAs and mRNA fusions entered meiotic tissues (anthers) and were fully active. In addition, we could show that mRNA transport is triggered by specific RNA motifs. Summarized our data suggest that small RNA molecules involved in epigenetic signaling and mRNAs are mobile and have the potential to enter meiotic cells. This suggests that a plant endogenous RNA-based signaling mechanism exists that allows RNA molecules produced in leaves to be allocated to spores, and consequently can be transferred to the progeny.

P 14| Hybrid necrosis in local populations of *Arabidopsis thaliana*

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Hybrid necrosis, a common type of hybrid incompatibility in plants, is suggested to have evolved as a by-product of natural selection for disease resistance. Recently, genes involved in hybrid necrosis in Arabidopsis thaliana have been identified to encode regulators or receptors of pathogen response. However, the role of hybrid necrosis in natural populations is still largely unknown. We have identified a case of hybrid necrosis among different parental lines collected around Tübingen, Germany, in 2007. Diallel crosses among the Tübingen individuals revealed several additional hybrids displaying necrotic symptoms. Based on genotyping, we showed that the necrosis phenotype is linked to a single ACD6 (ACCELERATED CELL DEATH 6) locus in chr 4. Additionally, we confirmed that ACD6 is both necessary and sufficient for the necrosis. The different individuals within populations showed allelic diversity at the ACD6 locus. They also revealed different phenotypic response. This indicates that ACD6, known to activate the immune response in Arabidopsis mutants, accessions and hybrids, is also causal for hybrid necrosis in local populations.

P 15| Genetic basis of F2 hybrid chlorosis in *Arabidopsis thaliana*

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Hybrid chlorosis has been assumed to act as a postzygotic reproductive barrier in many crop species. To investigate the underlying mechanisms, we study a case of F2 hybrid chlorosis in *Arabidopsis thaliana* present in a cross between the accessions Shahdara (Sha) from Tajikistan and Lovvik-5 (Lov-5) from Sweden. Especially the younger leaves of the chlorotic F2 hybrids show a lack of chlorophyll and an impairment of photosynthesis.
The F2 segregation (1:15) of chlorotic plants indicated that two recessively interacting loci are linked to the phenotype. Illumina based mapping showed that the two-recessive interacting genes were located in a 200 kb interval at the beginning of chromosome 1 (from Sha) and a twofold larger interval in the beginning of chromosome 5 (from Lov-5). We selected candidates for further analysis by comparing the non-synonymous SNPs in the coding sequence of the genes in the two mapping intervals. However, other mutations such as insertions, deletions or gene duplication cannot as yet be ruled out to be responsible for the phenotype. All candidate genes were silenced separately using an amiRNA approach in the parental lines and in the F3 line where the phenotype and the two causal regions were fixed. We are currently analyzing these amiRNA lines.
Finding out the mechanism behind F2 chlorosis in ShaXLov-5 and how environmental factors influence the phenotype will add to our understaning of epistatic interactions in *A. thaliana*.

P 16| Characterization of the plastidic *Oenothera* leaf shape mutant *pm45*

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The *plastome mutator* of *Oenothera* is a nuclear mutation conferring a high rate of plastome mutations. Generally the generated mutants show decrease in chlorophyll content and photosynthesis capacity. In previous screening a green line, *Cornell-1*, showing mutated chloroplast genome was isolated. After selfing *Cornell-1* a plastidic leaf shape mutant, named *pm45*, was identified.
In order to identify the polymorphism responsible for the leaf shape phenotype, the *pm45* and *Cornell-1* plastomes were sequenced. By aligning the two chloroplast genomes we have identified multiple INDELS in the 5´UTR and 5´end of *accD* gene coding for the β-carboxyl transferase (β–CT) subunit of the plastidial acetyl-coenzyme A carboxylase (ACCase). The enzyme catalyzes the first step of fatty acid biosynthesis. In dicots the plastidial ACCase is a heteromeric enzyme and AccD is the only subunit encoded in the chloroplast genome. AccD has been shown to be essential in *Nicotiana tabacum.* The catalytic domain is located in C-terminal of the subunit and is highly conserved. Conversely the N-terminal domain is least conserved and its function remain still unknown. Our data we could explain the *pm45* phenotype by a down regulation of the AccD subunit due to multiple INDELS in the promoter region or a destabilization of the complex due to INDELS in the N-terminal domain.

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P 17| NAC transcription factor EARLY QUIT controls senescence by affecting ethylene biosynthesis in *Arabidopsis thaliana*

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Leaf senescence is a highly complex, genetically programmed process that defines the final phase of leaf development. Onset and progression of leaf senescence are accompanied by dramatic changes in cellular metabolism. Many members of the NAC transcription factor (TF) family are known to show enhanced expression during senescence. We started to functionally characterize some selected NAC TFs to study their role for plant senescence. One of them we dubbed EARLY QUIT (EQT) due to the fact that its overexpression accelerated senescence in *Arabidopsis thaliana*, while knocking out its function delayed senescence. DAB and NBT staining revealed an over-accumulation of reactive oxygen species (ROS) in CaMV 35S overexpressors, while ROS formation was reduced in an *eqt* knock-out mutant compared to wild type. To investigate the regulatory network downstream of EQT, we determined its preferred binding site by BSSA (binding site selection assay) and performed microarray-based expression profiling using estradiol-inducible *EQT* overexpression lines. Our studies identified several putative direct target genes, including *ACC OXIDASE 5 (ACO5)*, which encodes the ethylene forming enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase. Transactivation assays using Arabidopsis mesophyll cell protoplasts confirmed EQT to be an upstream activator of *ACO5*, and immunoblot analysis revealed an over-accumulation of ACC oxidase protein upon estradiol induction. EQT binds to the *ACO5* promoter, as shown by electrophoretic mobility shift assay (EMSA) and ChIP-PCR. *EQT* expression itself is triggered by treatment with ACC (the ethylene precursor), indicating a positive feedback loop. We conclude that *EQT* controls a senescence regulatory pathway that triggers ethylene formation through activation of *ACC OXIDASE 5* and additionally impinges on ROS formation during senescence.

P 18| Functional analysis of cytokinin regulated *ERF* transcription factors in *Arabidopsis thaliana*

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The *Arabidopsis* ethylene-response factor genes (*AtERFs*) form a large gene family encoding plant-specific transcription factors regulating a number of developmental processes. They are also important for adaption to various biotic and abiotic stresses, and they can be induced by plant hormones. Previous published microarray studies (e. g. Brenner et al., 2005) revealed that a couple of *AtERF* genes are regulated by cytokinin. Due to their close phylogenetic relationship four *AtERF* genes (*ERF#102, ERF#103, ERF#104* and *ERF#105*) were chosen for a more detailed characterization. Based on phylogenetic analyses of the AP2/ERF-superfamily by Nakano et al. (2006) *ERF#102* to *ERF#105* are members of the group IXb.
The objective of this study was to analyze in more detail the changes in transcript abundance of the four *AtERF* genes after treatment with cytokinin using quantitative real-time PCR (qRT-PCR) and to study the consequences of gene knockout/knockdown mutations as well as gene overexpressions. qRT-PCR analyses confirmed *ERF* transcript regulation by cytokinin. Cytokinin sensitivity tests indicated that *ERF#102* to *ERF#105* could be involved in regulating root elongation in response to cytokinin. Further work investigated a regulation of *ERF#102* to *ERF#105* by other hormones and an importance in various abiotic stresses.

P 19| Unraveling the gene regulatory network of a senescence-associated NAC transcription factor in *Arabidopsis thaliana*

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Transcription factors (TFs) play a pivotal role for the control of leaf senescence, their precise molecular functions and integration into metabolic and signaling networks, however, remains largely unknown so far. Studies from several groups including ours have shown that many TFs of the WRKY, NAC and other families are upregulated during leaf senescence in Arabidopsis thaliana and other plant species. Here we present the results of a NAC TF, tentatively called JARF, for which a role in senescence has not been reported previously. To identify downstream target genes of JARF we expressed it in transgenic plants under the control of an estradiol-inducible promoter and tested global expression patterns shortly (3-5 h) after JARF induction, using Affymetrix ATH1 micro-arrays. We observed several genes related to jasmonic acid homeostasis to be affected by the TF, several of which harbor the JARF binding site in their promoters. To identify the developmental patterns of JARF expression and the impact of environmental factors on this, we generated JARF promoter:GUS transgenic lines. Promoter deletion studies identified highly conserved sequences in the JARF promoter. Results will be presented.

P 20| Unraveling the interactions between plants' plasma membrane microdomains and the cytoskeleton elements

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In this work we try to evaluate the interactions between the cytoskeleton and plants´ plasma membrane microdomains which are defined as small and dynamic entities in size of 20 to 100 nm, enriched in sterols, sphingo- and phospholipids and GPI-anchored proteins. They are involved in many cellular processes such as cell signaling, protein sorting and membrane trafficking.
In our experiments we used *Arabidopsis thaliana* cell suspension cultures which were treated with different concentrations of the actin and microtubular depolimerizating agents, Cytochalasin D and Oryzalin respectively. Subsequently, detergent resistant membranes (DRM), which are thought to correspond to the biological sterol-enriched membrane microdomain, were extracted and analyzed by LC/MS/MS system.
MaxQuant was used as a tool for peptide identification and label free quantification. Obtained peptides´ list were analyzed by cRacker (http://cracker.mpimp-golm.mpg.de/) which is an in-house application based on R for the statistical analysis of mass spectrometry measurements.
From a list of proteins which respond to the applied treatment we selected proteins which are considered as a plasma membrane microdomains´ constituents and are also sterol dependent. Among them we can find such proteins like ATPases, phosphate or sugar transporters which we then choose for the enzymatic activity measurements and GFP-studies with the use of confocal microscopy.
We detected a group of putative constituents of DRMs which respond to perturbations in the structure of the actin and microtubular cytoskeleton. This is an indication that in plants the localization of certain proteins in the sterol enriched microdomains is dependent on the interaction with cytoskeleton. Altered localization to microdomains can result in drastic changes of the activity of these proteins.

P 21| An Ensemble Approach to Dissect Plant Regulatory Sequences

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Given recent advances in sequencing technology, acquiring a catalog of all expressed genes is becoming increasingly easy [1]. However, determining the regulatory networks that drive gene expression, in different physiological, developmental or environmental conditions, is to date still a laborious task. Essential for elucidating such gene regulatory networks is the detection of functional cis-acting elements, such as transcription factor binding sites, in the vicinity of expressed genes.
The availability of full genome sequences allows upstream regions of co-expressed genes to be mined for reoccurring motifs, which are likely to be bound by proteins; therefore various tools have been developed to find cis-regulatory elements based on overrepresentation of motifs. We have evaluated the performance of three recently published tools (MEMESuite [2], MotifSuite [3] and DECOD [4]) on plant data. In line with previous findings, each tool predicts a complementary set of regulatory motifs.
Based on these findings, an automated workflow was developed, which makes use of a combination of these tools to improve detection of putative cis-regulatory elements. Furthermore, using comparative genomics, the phylogenetic conservation of the found motifs is evaluated. Finally, the merits of this workflow are shown on plant datasets.

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P 22| Nitrogen sparing mechanisms in *Chlamydomonas reinhardtii*

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Microalgae recently gained attention for producing high amounts of biofuels directly from CO_2 and sunlight during growth-inhibiting nutrient deficiencies, including nitrogen (N) deprivation [1]. N however is a key resource for all organisms and accordingly there are conserved mechanisms of adaptation to limiting environments.
In order to understand the acclimation of the metabolism to N limitation we analyzed transcriptomes (RNAseq) and proteomes of the model alga *Chlamydomonas reinhardtii* within 48 hours of N deprivation. In the absence of N in the medium, abundant dispensable proteins provide N to support growth. Total protein content was reduced, especially proteins with relatively higher N content were depleted while induced proteins contained less N than average, resulting in adapted cells with an increased C/N ratio in proteins and in total biomass compared to N-replete cells. Cells focused on heterotrophic growth as RNA and proteins in photosynthesis decreased while mitochondrial respiration

was unaffected. Accordingly, RNAs and proteins involved in chlorophyll biosynthesis decreased early and chlorophyll degradation was induced, resulting in total reduction of chlorophyll/cell. Additionally, proteins and RNAs of Calvin-Benson cycle enzymes were reduced, related metabolite pools increased. Ribosomes were depleted, but interestingly only for chloroplast ribosomes control took place at the transcript level. Finally, spared N was used to induce transporters and metabolic enzymes for various alternative N sources reflecting the soil environmental niche of this organism.

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P 23| Systems-level analysis of N-starvation induced TAG accumulation in a Chlamydomonas starchless mutant defective in ADP-glucose pyrophosphorylase

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To understand the molecular basis underlying increased triacylglycerol accumulation in starch-less Chlamydomonas mutants, we undertook comparative time course transcriptomics of *sta6* (CC-4348), a *cw* strain purported to represent the parental *STA6* strain (CC-4349), and 3 independent *STA6* strains (*STA6*-C2, *STA6*-C4 and *STA6*-C6, generated by complementation, CC-4565, -4566 and –4567) in the context of nitrogen deprivation. Despite the N-starvation induced dramatic remodeling of the transcriptome, there are relatively few differences between *sta6* vs. *STA6*, the most dramatic of which were in the abundance of transcripts encoding key regulated or rate-limiting steps in central carbon metabolism, specifically *ICL1*, *MAS1*, *TAL1*, *FBP1* and *PCK1*, suggestive of increased flux towards hexose-phosphate in *sta6* by up-regulation of the glyoxylate pathway and gluconeogenesis. Enzyme assays validated the increase in isocitrate lyase and malate synthase activities and targeted metabolite analysis with increased succinate and malate and decreased fructose-1,6-bisphosphate document the impact of these changes. The 3-fold increase in hexose-phosphate may result from a block in ADP-Glc synthesis and / or from these metabolic changes. The increased flux through the oxidative phase of the pentose phosphate pathway suggested by higher expression of *TAL1* may increase the intracellular NADPH pool in *sta6*, enabling greater TAG accumulation. Comparisons of the independent datasets on multiple strains allowed the delineation of a sequence of events in a global N-starvation response in Chlamydomonas, starting with up-regulation of alternative N assimilation routes and carbohydrate synthesis within minutes, followed by gametogenesis, and subsequently a more gradual up-regulation of genes encoding enzymes of TAG synthesis. In parallel, transcripts encoding photosynthesis components decreased rapidly. Finally, genome re-sequencing analysis indicated that i) the deletion in *sta6* extends into the neighboring gene, and ii) a commonly-used *STA6* strain (CC-4349 as well as the sequenced reference (CC-503) are not congenic with respect to *sta6* (CC-4348), underscoring the importance of using complemented strains for more rigorous assignment of phenotype to genotype.

P 24| Cyanobacterial metabolite profiles of the Golm Metabolome Database

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Metabolomics, the quantitative estimation of cellular metabolites, is well suited to investigate cyanobacterial metabolism and its adaptive response to various environmental conditions. Metabolomics also allows the phenotyping of changes in the cyanobacterial system caused by mutations.
The increasing amounts of metabolomic primary data and accompanying metadata of experimental descriptions require efficient electronic data handling and processing as are provided by data bases such as the Golm Metabolome Database (GMD, http://gmd.mpimp-golm.mpg.de/). GMD hosts original datasets of metabolite profiles from cyanobacteria and publication supplements. Information on metabolic reprogramming by mutations and different experimental conditions are efficiently combined and compared by a database such as the GMD to improve our knowledge and understanding of the cyanobacterial system.
The open GMD framework also allows the implementation of future experimental designs in the sense of a long term public repository. GMD initially focuses on GC-MS based metabolite profiling as a core metabolomic technology and can be extended to host additional and new analytical techniques to extend our current picture of cyanobacterial metabolism.

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P 25| Identification of a Mn transporter involved in Fe homeostasis under Fe deficiency in *Arabidopsis thaliana*

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Iron (Fe) is an essential element for plant nutrition. In case of insufficient Fe uptake, plants enhance expression of the Fe transport protein IRT1 (iron-regulated transporter 1). IRT1 was shown not only to transport Fe but also other heavy metals incl. Mn, Co, Zn and Cd. This has been supported by the observation that the excess accumulation of those metals is strongly reduced in Fe-deficient *irt1* mutant plants. Since free heavy metals can cause oxidative stress, plants have evolved mechanisms to detoxify heavy metals. Accordingly, AtMTP3, which is localised in the tonoplast and co-regulated with IRT1, confers Zn and Co tolerance under Fe deficiency. However, little is known about how plants detoxify Mn under Fe deficiency.

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P 26| Identification and characterization of thermomemory-transcriptional regulators

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Plants have evolved a molecular memory that helps them to better cope with stressful events after having experienced a previous environmental stress. *Arabidopsis thaliana* Col-0 seedlings treated with a prior moderate (and non-lethal) temperature regime (37°C, 90 min; so-called priming) exhibit improved response to a future high-temperature stress (45°C, 60 min). To identify transcription factors (TFs) associated with thermomemory we used quantitative real-time PCR (qRT-PCR) to test the expression of 1,880 TFs in primed seedlings 2, 4 and 28 h after the priming stimulus, and compared it to unprimed (non-treated) seedlings. One of the TFs, namely *JUNGBRUNNEN1* (*JUB1*), showed elevated expression even 28 h into the memory phase; JUB1 is a NAC TF that positively regulates longevity in Arabidopsis. *JUB1* overexpression enhances heat stress tolerance in primed and unprimed conditions, whereas *jub1-1* knockdown lines show impaired thermomemory and thermotolerance compared to wild-type plants. The thermomemory-related expression of *JUB1* resembles that of the well-known thermomemory genes *HSFA2*, encoding a heat shock TF, and *HSA32*, encoding a heat shock protein. Our analysis also identified eight further TFs showing altered expression during the thermomemory phase, revealing them as new candidates for studies to decode the molecular processes controlling thermomemory.

Supplements

to the

Printed Abstract Book

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Hannes Link studied Chemical Engineering at the Technical University Munich, Germany. During his studies, he looked at the experimental optimization of bioprocesses and developed a MATLAB/Simulink-based tool for analysis and optimization of biochemical systems. He stayed at the TU Munich to do his PhD with Professor Weuster-Botz, working on metabolic control analysis of fed-batch fermentation processes. Hannes Link received his doctorate in 2009. After a short time as postdoc in Prof. Weuster-Botz's lab, he moved to Switzerland. He is a postdoc in Prof. Uwe Sauer's lab at the ETH Zurich since March 2010. His research focusses on the identification and *in vivo* function of allosteric metabolite-protein interactions.

Abstract

Inferring metabolic feedback regulation from dynamic metabolite data

Existing maps of protein-protein and protein-DNA interactions do not comprehensively represent the dynamic molecular interactions that govern phenotypic behavior, in part because interactions between proteins and metabolites are missing. In the Sauer lab we investigate protein-metabolite interactions in order to understand their function for metabolic feedback regulation. The key input in these studies are dynamic metabolite data generated either by targeted quantitative metabolomics [1] or untargeted metabolome profiling [2].

Recently, we investigated the reversal of the glycolytic pathway for 30 seconds by tracing intracellular fluxes and quantitation of absolute metabolite concentrations in the bacterium *Escherichia coli* [3]. The metabolite dynamics were predicted with a kinetic model of glycolysis, allowing to systematically test the effect of 126 putative allosteric protein-metabolite interactions. This approach could identify metabolic feedback that is required for a reversible switch between gluconeogenesis and glycolysis.

Our current effort is to find metabolic feedback that actively regulates biosynthetic pathways. Therefore we monitored the response of a starving *E. coli* culture to glucose using automated flow injection into the mass spectrometer. The method allows to monitor the response of more than 300 metabolites over 30 minutes with a sampling frequency of 15 seconds. These data show that several of the known regulatory metabolites actively regulate amino acid and purine synthesis.

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P 27| STAX, a novel negative transcriptional regulator of Arabidopsis leaf senescence

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Leaf senescence is a highly regulated, systematic process with great impact on yield, biomass and nitrogen partitioning. The process is basically mediated by developmental age; however, it is additionally influenced by an array of internal and environmental signals. Delayed senescence, accompanied by an extended period of photosynthesis, is often coupled with elevated stress tolerance and/or higher biomass accumulation. Thus, the timing of senescence is crucial in determining crop yield, having great agricultural importance. Our group studies the function of senescence-associated transcription factors (TFs) in order to unravel the complex regulatory mechanisms underlying the onset and progression of leaf senescence.
Recently, we identified a novel TF, called STAX, as a major regulator of leaf senescence in Arabidopsis thaliana. STAX expression is enhanced during age-dependent as well as dark- and salt-induced senescence. Overexpression of STAX results in extended life span, whereas its knock-out mutant shows accelerated senescence suggesting a negative regulatory role for STAX on leaf senescence. In addition to delayed senescence, STAX overexpressors displayed a significant delay in bolting and increase in leaf biomass. In order to understand the gene regulatory network controlled by STAX, its binding site was identified. Using estradiol-induced overexpression of the STAX TF in combination with microarray-based transcriptome profiling (using Affymetrix ATH1 arrays) we were able to identify genes rapidly responding to enhanced STAX expression, representing candidate direct target genes. Taken together, our data suggest STAX as a key regulator of plant growth and development, including leaf senescence.

P 28| Plastid mediated speciation barriers in the evening primrose (*Oenothera*)

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The model plant *Oenothera* (evening primrose) is an organism perfectly suited to study molecular cause and evolutionary driving forces, connected with cytoplasmic speciation barriers. Crosses within the genus often result in so-called plastome-genome incompatible (PGI) offspring. This plastid mediated form of hybrid bleaching acts a speciation barrier under the Dobzhansky-Muller model of reproductive isolation.
Within Oenothera genus three basic nuclear genomes (A,B and C) that can occur as a homozygous and as a heterozygous constitution 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 of them exist in nature. The remaining 18 combinations display PGI to various degrees and can occur naturally as inviable hybrids.

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In a pilot study the incompatible combination AB-I was chosen to identify molecular determinants causing PGI in *Oenothera*. This incompatibility builds a strong hybridization barrier between AA-I and AB-II/AB-III species. It appears, that plastome type I is incompatible in the AB background, but the combination AB-II, AB-III, and AB-IV remain green. Sequence comparison of these four plastomes unveils a specific deletion of 148 bp in plastome I, which is not present in plastomes II- IV, turning this deletion to an appealing candidate locus. The deletion affects the divergently operating promoter region between the *psbB* gene, a core subunit of photosystem II and the *clpP* gene, encoding a subunit of plastid ATP-dependent protease. Physiological and molecular analyses substantiate the relevance of this locus causing PGI in AB-I *Oenothera* plants. These results point to the fact that altered gene regulation of plastid genes may play a role in the microevolution of the genus probably as mechanism of adaptation to changing environmental conditions. in plastomes II- IV, turning this deletion to an appealing candidate locus. The deletion affects the divergently operating promoter region between the *psbB* gene, a core subunit of photosystem II and the *clpP* gene, encoding a subunit of plastid ATP-dependent protease. Physiological and molecular analyses substantiate the relevance of this locus causing PGI in AB-I *Oenothera* plants. These results point to the fact that altered gene regulation of plastid genes may play a role in the microevolution of the genus probably as mechanism of adaptation to changing environmental conditions.

P 29| Integrative analysis of Transcriptome, Translatome, and Proteome data from root cells of *Arabidopsis thaliana*

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