



Plant Biology of the Next Generation

- **Conference of the SFB924**
"Molecular mechanisms regulating yield
and yield stability in plants."
- **September 18 – 20, 2013**
Wissenschaftszentrum Weißenstephan
Technische Universität München



- **Marc Albertsen** (Pioneer, Johnston, USA)
- **Brenda Andrews** (University of Toronto, Canada)
- **Eva Bauer** (Technische Universität München, TUM, Germany)
- **Chris Bowler** (ENS Paris, France)
- **John Doebley** (University of Wisconsin-Madison, USA)
- **Thomas Dresselhaus** (University Regensburg, Germany)
- **Pascal Falter-Braun** (TUM, Germany)
- **Monika Frey** (TUM, Germany)
- **Wolf Frommer** (Carnegie, Washington, USA)
- **Alfons Gierl** (TUM, Germany)
- **Christine Gietl** (TUM, Germany)
- **Ulrich Hammes** (University of Regensburg, Germany)
- **Bin Han** (Shanghai Institutes for Biological Sciences, CAS, China)
- **Ikuko Hara-Nishimura** (Kyoto University, Japan)
- **Tetsuya Higashiyama** (University of Nagoya, Japan)
- **Ralph Hückelhoven** (TUM, Germany)
- **Dirk Inzé** (VIB Ghent, Belgium)
- **Erika Isono** (TUM, Germany)
- **Frank Johannes** (University of Groningen, NL)
- **Thomas Lahaye** (LMU, München, Germany)
- **Cathie Martin** (John Innes Centre, UK)
- **Klaus F.X. Mayer** (Helmholtz Zentrum München, Germany)
- **Thomas Ott** (LMU, München, Germany)
- **Martin Parniske** (LMU, München, Germany)
- **Brigitte Poppenberger** (TUM, Germany)
- **Salomé Prat** (CSIC Madrid, Spain)
- **Kay Schneitz** (TUM, Germany)
- **Chris-Carolin Schön** (TUM, Germany)
- **Waltraud Schulze** (MPI Potsdam-Golm, Germany)
- **Wilfried Schwab** (TUM, Germany)
- **Claus Schwechheimer** (TUM, Germany)
- **Stephanie Sprunck** (University of Regensburg, Germany)
- **Cristobal Uauy** (John Innes Centre, UK)
- **Corina Vlot-Schuster** (Helmholtz Zentrum München, Germany)
- **Ry Wagner** (Dow Agrosience, Indianapolis, USA)
- **Dani Zamir** (Hebrew University, Israel)



HelmholtzZentrum münchen
Deutsches Forschungszentrum für Gesundheit und Umwelt



Conference venue (Please note the modification.)

Lecture Hall 12/Hörsaal 12

Wissenschaftszentrum Weihenstephan

Technische Universität München

Emil Ramann Straße 2

85354 Freising-Weihenstephan

WLAN

Network: SFB924_CONFERENCE-Sept2013

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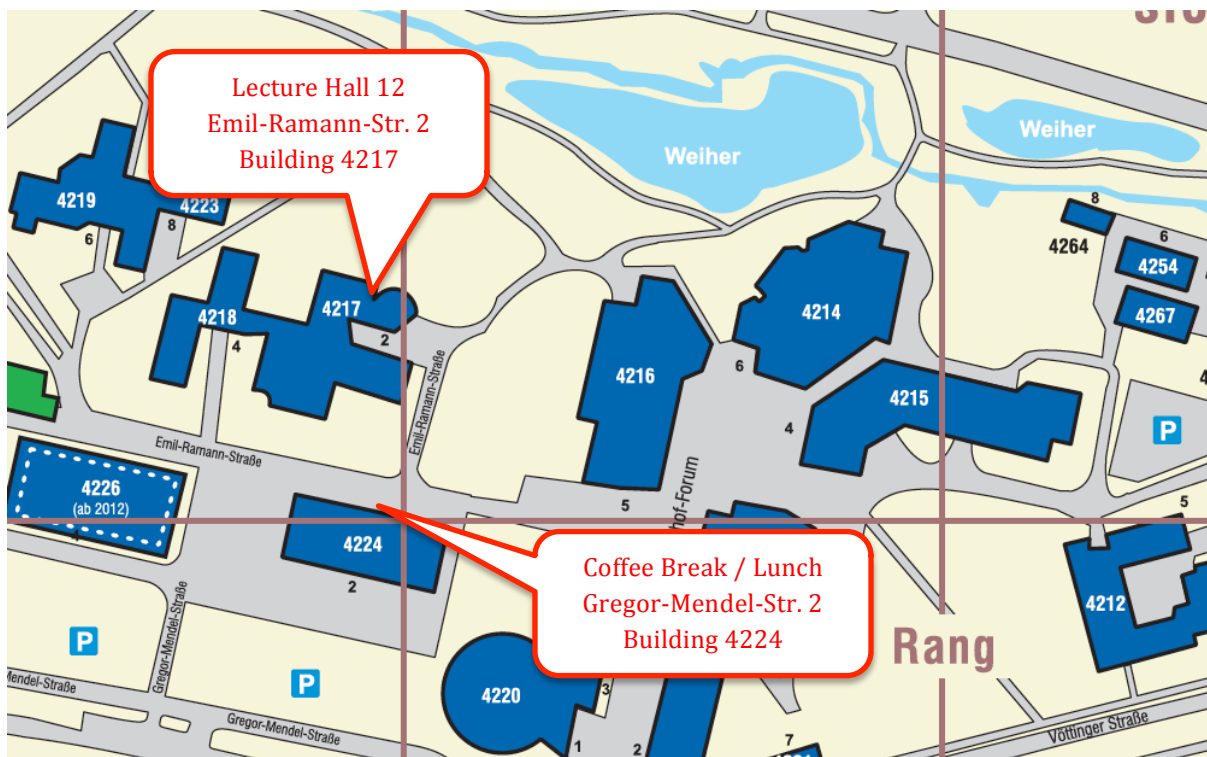
Hotel Bayrischer Hof (+49) (0)8161 538300

Travel expenses

Invited speakers can download the claims form for travel expenses from the conference link at <http://sfb924.wzw.tum.de>

City and campus map

City and campus map (Please note the modification!)



Program

Wednesday September 18, 2013

12:00 Start registration (*small snacks will be served*)

Session 1 Chair: Thomas Dresselhaus (Universität Regensburg)

14:00 Alfons Gierl (Dean of the Faculty)

Short welcome

Dirk Inzé (VIB Gent, Belgium)

Plant biology for an evergreen future (p.13)

14:30 Mathew Lewsey and Joe Ecker (Salk Institute, San Diego, USA)

Regulatory networks controlling hormone-mediated growth (p.14)

14:50 Claus Schwechheimer (Technische Universität München)

Regulation of plant growth by GATA transcription factors (p.15)

15:10 Alexander Christmann and Erwin Grill (Technische Universität München)

Control of the protein kinase GCA2 on ABA signaling (p.16)

15:30 Svenja Rademacher (Technische Universität München)

Genetic architecture of stable carbon isotope discrimination in maize (p.17)

15:45 Stefanie Ranf (Technische Universität München)

Characterization of calcium signaling mutants in Arabidopsis innate immunity (p.18)

16:00 Kay Schneitz (Technische Universität München)

Linking receptor-like kinase signaling and plasmodesmata conductivity during tissue morphogenesis in *Arabidopsis thaliana* (p.19)

16:20 Pecha kucha PK1 (One-slide poster presentations) (p.20-21)

16:30 Coffee break and posters

(Wednesday September 18, 2013)

Session 2 Chair: Thomas Ott (LMU Munich)

17:00 Ry Wagner (Agrinos, Lysaker, Norway)

New insights to increased crop yield: Focusing on the importance of the microbiome (p.22)

17:20 Ulrich Z. Hammes (Universität Regensburg)

Amino acid supply of sink tissues (p.23)

17:40 Martin Parniske (LMU Munich)

Signal transduction in plant root symbiosis (p.24)

18:00 Caroline Gutjahr (LMU Munich)

INHOSPITABLE is required for early steps of arbuscular mycorrhiza development (p.25)

18:15 Corina Vlot-Schuster (HMGU München-Neuherberg)

Bacteria trigger systemic acquired resistance in the cereal crop barley (p.26)

18:35 Orlando di Lange (LMU Munich)

Breaking the DNA binding code of *Ralstonia solanacearum* TAL effectors provides new possibilities to generate plant resistance genes against bacterial wilt disease (p.27)

18:55 Pecha kucha PK2 (One-slide poster presentations) (p.28-29)

afterwards Mixer with drinks and food

Thursday September 19, 2013

Session 3 Chair: Caroline Gutjahr (LMU Munich)

9:00 Ikuko Hara-Nishimura (Kyoto University, Japan)

Intracellular machineries for movement of endoplasmic reticulum and nucleus in *Arabidopsis thaliana* (p.30)

9:30 Ralph Hückelhoven (Technische Universität München)

RAC/ROP GTPase signalling in microtubule organization and susceptibility of barley to powdery mildew (p.31)

9:50 Christine Gietl (Technische Universität München)

The role of KDEL-tailed cysteine endopeptidases in programmed cell death of plants (p.32)

10:10 Erika Isono (Technische Universität München)

The deubiquitinating enzyme AMSH and ESCRT-III are required for intracellular trafficking and autophagic degradation in *Arabidopsis thaliana* (p.33)

10:30 Brigitte Poppenberger (Technische Universität München)

Interplay of phosphorylation and SUMOylation events determines CESTA protein fate in brassinosteroid signaling (p.34)

10:50 Pecha kucha PK3 (One-slide poster presentations) (p. 35-36)

11:00 Coffee break and posters

(Thursday September 19, 2013)

Session 4 Chair: Corina Vlot-Schuster (HMGU München-Neuherberg)

11:30 Marc Albertsen (Pioneer Hi-Bred, Johnston, USA)

Enhancing crop productivity through manipulation of reproductive biology
(p.37)

11:50 Thomas Dresselhaus (Universität Regensburg)

Signaling during pollen tube perception (p. 38)

12:10 Stefanie Sprunck (Universität Regensburg)

Posttranslational regulation of sperm-activating EC1 proteins (p.39)

12:30 Tetsuya Higashiyama (Nagoya University, Japan)

Live-cell study in combination with synthetic chemistry: pollen tube
guidance as a model (p.40)

12:50 Thomas Ott (LMU Munich)

A cell biological survey of the heterogeneous membrane micro-
domain landscape of plant cells (p.41)

13:10 Pecha kucha PK4 (*One-slide poster presentations*) (p.42-43)

13:20 Lunch break and posters

(Thursday September 19, 2013)

Session 5 Chair: Stefanie Ranf (Technische Universität München)

15:00 John Doebley (University of Wisconsin-Madison, USA)

The genetic architecture of maize domestication: Low hanging fruit and dark matter (p.44)

15:30 Bin Han (Chinese Academy of Sciences, Shanghai, China)

Sequencing diverse rice germplasms for GWAS of complex traits and rice domestication study (p.45)

15:50 Chris-Carolin Schön (Technische Universität München)

Genome-enabled prediction of complex phenotypes – advances in quantitative genetics (p.46)

16:10 Salomé Prat (CSIC, Madrid, Spain)

FT control of potato storage organ formation: new prospective to the selection of heat-stress cultivars (p.47)

16:30 Coffee break and posters

(Thursday September 19, 2013)

Session 6 Chair: Ulrich Z. Hammes (Universität Regensburg)

17:10 Dani Zamir (The Hebrew University of Jerusalem, Rehovot, Israel)

Where have all the plant phenotypes gone? (p.48)

17:30 Klaus F.X. Mayer (HMGU München-Neuherberg)

Hosting three genomes: the bread wheat genome and its transcriptional interplay during grain formation (p.49)

17:50 Cristobal Uauy (John Innes Centre, Norwich, UK)

The application of molecular genetics for sustainable wheat production (p.50)

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

Conference dinner at the Hofbräuhaus München

Return ca. 23:00

Friday September 20, 2013

Session 7 Chair: Klaus F.X. Mayer (HMGU München-Neuherberg)

9:00 Brenda Andrews (University of Toronto, Canada)

From phenotypes to pathways: global exploration of cellular networks using yeast functional genomics (p.51)

9:30 Pascal Falter-Braun (Technische Universität München)

Convergent targeting of a validatable host-network by pathogens from three kingdoms of life (p.52)

9:50 Waltraud Schulze (MPI Potsdam-Golm and University Hohenheim)

From phosphoproteomics networks to new functions in plant receptor kinases (p.53)

10:10 Chris Bowler (ENS Paris, France)

The *TARA*-Oceans project: towards plankton eco-systems biology (p.54)

10:30 Frank Johannes (University of Groningen, NL)

Mapping the transgenerational epigenetic basis of complex traits (p.55)

10:50 Coffee break

(Friday September 20, 2013)

Session 7 Chair: Thomas Lahaye (LMU Munich)

11:20 Cathie Martin (John Innes Centre, Norwich, UK)

Engineering phenylpropanoid metabolism for healthier foods (p.56)

11:40 Wolf B. Frommer (Carnegie Institution, Stanford, CA)

Quantitative imaging of transport activity and metabolite dynamics with fluorescent biosensors (p.57)

12:00 Wilfried Schwab (Technische Universität München)

A peroxidase regulates the flux to anthocyanins and lignin in *Fragaria x ananassa* fruit (p.58)

12:20 Monika Frey (Technische Universität München)

Comparative analysis of benzoxazinoid biosynthesis in monocots and dicots (p.59)

12:40 Claus Schwechheimer (Technische Universität München)

Concluding remarks

- End of the conference (*small snacks available*) -

Talks

Abstracts

-

Pecha Kucha

Poster titles

Plant biology for an evergreen future

Dirk Inzé

Department of Plant Systems Biology, VIB, UGent, Belgium

Plants support almost all life on earth. By using very efficiently sunlight as energy source to capture carbon dioxide and to build sugars, plants have an indispensable role in providing ecosystems with energy and chemical building blocks. It is estimated that photosynthesis produces 100 billion tons of dry weight annually, only a small part of which is used for feeding mankind. Nevertheless, to keep our planet viable, we should not increase the amount of areal land for food production and consequently, providing the still exponentially increasing world population with sufficient calories and nutrients will require further gains in crop yield. In this lecture, I will illustrate with my own work on plant leaf size how an integrated and system biology driven approach using research performed on both model systems (*Arabidopsis*) as well as crops (maize) can generate novel insights and strategies in how to improve agricultural productivity. This type of translational research is much facilitated by disruptive technologies including NGS, affinity-based interactomics and automated phenotyping. Further implementing these technologies for field grown (transgenic) crops will be a pivotal component of future success and will require a drastic change in policy towards field trials. Ultimately plant research will enable plant biologists and breeders to develop the next generation of crops which, in combination with advances in agricultural management, will allow for producing more and better crops while reducing the environmental footprint and addressing key problems such as climate change, drought tolerance, water scarcity and tolerance to new threatening diseases. As this new green revolution has to be sustainable, I named it 'evergreen revolution'.

Regulatory networks controlling hormone-mediated growth

Lewsey M.G.^{1,2}, Song L.^{1,2}, Huang S.C.^{1,2,3}, Xie M.^{1,2}, Zander M.^{1,2}, Chang K.N.^{1, 2}, Wanamaker S.^{1,2}, O'Malley R.C.^{1,2}, Weirauch M.T.^{4,5}, Hughes T.R.⁴, Briggs S.P.⁶, Krogan N.J.⁷, Bar-Joseph Z.⁸ and Joe R. Ecker^{1,2,3}

¹ Plant Biology Laboratory; ² Genomic Analysis Laboratory; ³ Howard Hughes Medical Institute, The Salk Institute for Biological Studies, La Jolla, CA, USA.

⁴ Department of Molecular Genetics and Banting and Best Department of Medical Research, University of Toronto, ON, Canada.

⁵ Division of Rheumatology and the Center for Autoimmune Genomics and Etiology (CAGE), Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

⁶ Section of Cell and Developmental Biology, University of California, San Diego, La Jolla, CA, USA.

⁷ Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, CA, USA.

⁸ Lane Center for Computational Biology, School of Computer Science, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, PA 15213, USA.

The response of organisms to their environment is a consequence of biological processes that change gene expression and protein activity. These regulatory processes involve the coordinated function of many genes and proteins, integrating inputs from multiple signaling pathways. Hence, genes and proteins are not independent units; rather their actions are deeply interconnected, influencing each other in their activity and regulation. Experimental approaches that combine different types of data from different types of experiments can be a very powerful way to gain new insights into the gene networks that control these processes. Plant science is now at the crossroads of being able to harvest such data for all the genes in a genome. We are create a multi-layered map of plant growth networks for the hormones abscisic acid, auxin, brassinosteroid, cytokinin, ethylene (ET), jasmonate, and salicylic acid, utilizing genome-scale techniques. Our initial objective is to generate a transcriptional regulatory network of hormone responses in the etiolated Arabidopsis seedling. We have selected 200 transcription factors (TFs) across these hormone-signaling pathways for investigation. The genes targeted by these TFs are being identified, genome-wide, using chromatin immunoprecipitation sequencing of tagged TFs under native expression. Our initial data from TFs of the jasmonate, ET, brassinosteroid, abscisic acid and cytokinin signaling pathway indicate that a core set of highly connected targets exists, bound by TFs from multiple pathways. However, each TF also has a set of unique targets. To increase coverage to TFs not analyzed by ChIP-Seq, three complimentary techniques are being used. Firstly, we are identifying regions of open chromatin by DNase hypersensitivity sequencing. Secondly, protein binding microarrays have been used to characterize the DNA motifs bound by hundreds of Arabidopsis TFs. Thirdly, the binding preferences of TFs within genomic DNA are being examined *in vitro* by a novel immunoprecipitation assay. By scanning the DNA sequence underlying regions of open chromatin for TF binding motifs, we will be able to predict which TFs are binding in those regions. The regulatory consequences of TF binding events are being examined both at the transcript and protein level. High-resolution time series transcriptome data has revealed cross-regulation of multiple hormone response pathways by the ET-responsive TF EIN3. Protein abundance, modification and complex formation are being assayed by proteomic and phospho-proteomic studies of hormone responses. Furthermore, we have employed two methods to investigate protein-protein interactions; a novel yeast two-hybrid assay coupled to high-throughput sequencing, enabling the simultaneous assay of hundreds of protein-protein interactions; and a novel microarray-based technique to screen individual proteins for interactions with a library of thousands of target proteins. An interactome of unparalleled coverage will be generated from these datasets. Our ultimate goal is to integrate these diverse data into a multi-layered network, allowing us to identify the manner and mechanisms through which hormone signals are integrated to result in coordinated seedling growth.

GATA transcription factors integrate signaling downstream from gibberellin, auxin and cytokinin signaling

René Richter, Carina Behringer, Emmanouil Bastakis, Quirin Ranftl, Melina Zourelidou, and **Claus Schwechheimer**

Plant Systems Biology, Technische Universität München, Freising-Weihenstephan, Germany

During the "Green Revolution", the introduction of growth regulatory traits interfering with the biosynthesis or signaling of the phytohormone gibberellin (GA) has resulted in substantial increases in wheat and rice yields. Research over the past decade has elucidated the underlying molecular mechanisms that govern GA-dependent growth regulation. Central regulators of GA signaling are the GA-labile DELLA repressors that inhibit, in the absence of GA, a surprisingly large and diverse set of transcription factors including the PHYTOCHROME INTERACTING FACTORS (PIFs).

In order to understand how GA controls plant growth, we have examined GA-regulated target genes at the genetic, molecular biological and physiological level. The two GATA family transcription factors GNC and GNL have emerged from these analyses as important regulators of seed germination, greening, flowering time as well as a number of other traits. *GNC* and *GNL* transcription is regulated directly downstream from DELLAs by the PIFs (1). However, *GNC* and *GNL* transcription is also regulated by AUXIN RESPONSE FACTOR2 (ARF2), a member of this transcription factor family that is not regulated by the auxin-labile Aux/IAA repressors (2). However, the abundance of ARF2 protein and hence ARF2 activity is enhanced by GA through a posttranscriptional mechanism suggesting that GA signaling can regulate normally auxin-regulated transcription targets to modulate auxin responses as part of a GA-auxin cross-talk. Further examples for a regulatory cross-talk on the regulation of *GNC* and *GNL* will be presented (3) as well as our advances to understand GNC and GNL function in other species and to identify their target genes.

¹ Richter R, Behringer C, Müller IK, Schwechheimer C. (2013) *Genes Dev.* 24(18):2093-104.

² Richter R, Behringer C, Zourelidou M, Schwechheimer C. (2013) *PNAS* 110:13192-7.

³ Richter R, Bastakis E, Schwechheimer C. *Plant Physiol.* (2013) 162:1992-2004.

Control of the protein kinase GCA2 on ABA signaling

Alexander Christmann, Arthur Korte, Christian Wellmann, Sophie Pleissner, Gereon Czap and Erwin Grill

Botany, Technische Universität München, Freising-Weihenstephan, Germany

The plant hormone abscisic acid (ABA) acts both as a developmental signal and as an integrator of environmental cues such as drought and cold. ABA perception recruits an ABA-binding regulatory component RCARs/PYR1/PYLs and an associated protein phosphatase 2C (PP2C) to generate a phosphatase-inactive heterodimer in the presence of ABA. PP2C inactivation permits autoactivation of SnRK2-type protein kinases, which are positive regulators of ABA responses such as control of gene expression, stomatal closure, and plant growth inhibition. Early steps of the ABA transduction pathway involve the integration of Ca^{2+} signals. Screening more than 200,000 chemically mutagenized Arabidopsis seedlings for ABA-insensitive root growth, we identified *gca2-1*. The *gca2-1* mutant is impaired in ABA responses but also in redox- and carbon dioxide-mediated control of stomatal aperture. The data support a role of GCA2 in the integration of endogenous and external signals. The *GCA2* locus has been isolated by positional cloning and identified to encode a calmodulin-domain-like protein kinase (CDPK). The characterisation of GCA2 and its interaction with the ABA signal pathway will be presented.

Genetic architecture of stable carbon isotope discrimination in maize

Svenja Rademacher¹, Sebastian Gresset¹, Peter Westermeier¹, Thomas Prestler², Milena Ouzunova², Peter Westhoff³ and Chris-Carolin Schön¹

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² KWS SAAT AG, 37555 Einbeck, Germany

³ Institute of Plant Molecular and Developmental Biology, Heinrich-Heine-University, 40225 Düsseldorf, Germany

Drought is one of the major constraints of plant productivity worldwide. For C3 plants the trait stable carbon isotope discrimination ($\Delta^{13}\text{C}$) has long been used to detect genetic differences for water use efficiency and has been shown to be a precise predictor of yield under drought conditions. In C4 plants, physiological mechanisms and genetic components influencing $\Delta^{13}\text{C}$ have not been described. To assess whether $\Delta^{13}\text{C}$ can also be used in C4 plants as an indirect selection trait it needs to be shown that $\Delta^{13}\text{C}$ is under genetic control. We analyzed a maize (*Zea mays* L.) introgression library derived from two elite parents to investigate whether $\Delta^{13}\text{C}$ is under genetic control in the C4 species maize. The Illumina MaizeSNP50 Bead Chip was used for high density genotyping and a detailed structural characterization of 77 introgression lines. Kernel $\Delta^{13}\text{C}$ as well as developmental traits and photosynthesis related parameters were measured in the field and in the greenhouse. We detected highly heritable significant genetic variation for $\Delta^{13}\text{C}$ under field and greenhouse conditions and several IL lines consistently differed from the recurrent parent regarding $\Delta^{13}\text{C}$ values within and across both phenotyping platforms. $\Delta^{13}\text{C}$ was significantly associated with 22 out of 164 analyzed genomic regions indicating a complex genetic architecture of $\Delta^{13}\text{C}$. Plant developmental stage did not have an effect on $\Delta^{13}\text{C}$ expression since phenotypic as well as genotypic correlations between $\Delta^{13}\text{C}$, flowering time and plant height were not significant. We show for the first time that $\Delta^{13}\text{C}$ is genetically regulated and exhibits a complex genetic architecture in the C4 species maize.

Characterization of calcium signaling mutants in *Arabidopsis* innate immunity

Stefanie Ranf¹, Justin Lee², Dierk Scheel²

¹ Wissenschaftszentrum Weihenstephan Technische Universität München, Institut für Phytopathologie, Emil-Ramann-Str. 2, D-85350 Freising-Weihenstephan, Germany

² Leibniz-Institut für Pflanzenbiochemie, Abteilung Stress- und Entwicklungsbiologie, Weinberg 3, D-06120 Halle (Saale), Germany

During attempted infection of plants, pathogens are betrayed by conserved “Microbe-Associated Molecular Patterns” (MAMPs) that are recognized by specific host receptors and initiate intracellular signalling cascades leading to MAMP-triggered immunity. Endogenous “Damage-Associated Molecular Patterns” (DAMPs) similarly elicit receptor-mediated defences. Rapid elevations in the free cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_{\text{cyt}}$) are a core component of MAMP and DAMP signal transduction and are crucial for establishment of downstream responses, such as reactive oxygen species (ROS) accumulation, activation of protein kinases, and induction of defence gene expression. The MAMPs flagellin (flg22), elongation factor Tu (elf18) and chitin, as well as the DAMP, AtPep1, provoke generally similar prolonged $[\text{Ca}^{2+}]_{\text{cyt}}$ elevations in *Arabidopsis thaliana* but with distinct lag times and amplitudes. Mutant analysis revealed a feedback impact of the Ca^{2+} -dependent ROS accumulation on the $[\text{Ca}^{2+}]_{\text{cyt}}$ elevation. Despite the pivotal role of Ca^{2+} as second messenger in MAMP signalling, only a few participating Ca^{2+} channels and transporters are known. Using chemically mutagenised *Arabidopsis* seedlings expressing the Ca^{2+} -reporter aequorin, we isolated mutants with *changed calcium elevation* (*cce*) in response to flg22. These comprise novel alleles of the flagellin receptor *FLS2* and the receptor-associated kinase *BAK1*, as well as other *cce* mutants with partially reduced or enhanced $[\text{Ca}^{2+}]$ elevations in response to several MAMPs and DAMPs. Thus, these *CCE* genes encode components shared by different MAMP/DAMP signalling pathways and will be useful to unravel early signalling events in plant-microbe interactions. Currently, we are identifying the *CCE* genes by mapping and genome sequencing and characterising their role in innate immunity.

Linking receptor-like kinase signaling and plasmodesmata conductivity during tissue morphogenesis in *Arabidopsis thaliana*

Prasad Vaddepalli¹, Anja Herrmann^{1,3}, Yashodar Babu^{1,4}, Lynette Fulton^{1,5}, Stefan Hillmer², David G. Robinson² and **Kay Schneitz**¹

¹ Plant Developmental Biology, Wissenschaftszentrum Weihenstephan, Technische Universität München, Emil-Ramann-Str. 4, 85354 Freising, Germany

² Plant Cell Biology, Centre for Organismal Studies, University of Heidelberg, Im Neuenheimer Feld 230, 69120 Heidelberg, Germany

³ Present address: Institute of Plant Biology, University of Zurich, Zollikerstr. 107, CH-8008 Zurich

⁴ Present address: Dept. of Cell Biology, Max Planck Institute of Developmental Biology, Spemannstr. 35-39, D-72076 Tübingen

⁵ Present address: School of Biological Sciences, Monash University, VIC 3800, Australia

Tissue morphogenesis in plants requires the coordination of cellular behavior within and across clonally distinct histogenic layers. In *Arabidopsis*, the leucine-rich repeat receptor-like kinase STRUBBELIG (SUB) is required for inter-cell-layer communication during floral and ovule development. SUB is an atypical receptor-like kinase that when mutated, shows defects in floral organ, stem and silique shape, ovule integument morphogenesis and root hair patterning. SUB affects tissue morphogenesis in a non cell-autonomous manner thus influencing the behavior of neighboring cells. *QUIRKY* (*QKY*), *ZERZAUST* (*ZET*) and *ANGUSTIFOLIA* (*AN*) belong to *STRUBBELIG-LIKE MUTANT* (*SLM*) class of genes, proposed to contribute to *SUB*-dependent signal transduction. In the present work, molecular characterization of *QKY* was undertaken with the main focus on its role in *SUB*-mediated signaling. Mapping and molecular identification of *QKY* revealed that it encodes a novel multiple C2 domain-containing transmembrane protein (MCTP). Biochemical studies imply that *QKY* binds to phospholipids in a Ca^{+2} -dependent manner. Protein localization experiments indicate that *QKY* is specifically associated with plasmodesmata (PD). Immunogold electron microscopy results confirm the PD localization of *QKY* and also reveal the previously unknown PD localization of SUB. SUB and *QKY* do not appear to be involved in non-selective movement GFP-sized proteins. Yeast-two-hybrid data indicate that SUB and *QKY* can interact directly. Thus, the data imply that SUB signaling mediates tissue morphogenesis by influencing selective transport of molecules through PD.

Pecha kucha - Session 1 (Cornelia Kolb, Technische Universität München)

PK1.1 Emmanouil Bastakis and Claus Schwechheimer (Plant Systems Biology, Technische Universität München)

Identification of target genes of the Arabidopsis GATA transcription factor GNL

PK1.2 Quirin Ranftl and Claus Schwechheimer (Plant Systems Biology, Technische Universität München)

Analysis of the GA-regulated GATA-transcription factors GNC/GNL and their paralogous genes

PK1.3 Ulrich Lutz and Claus Schwechheimer (Plant Systems Biology, Technische Universität München)

Natural variation of gibberellin-regulated flowering in *Arabidopsis thaliana*

PK1.4 Nina Lantzouni and Claus Schwechheimer (Plant Systems Biology, Technische Universität München)

Gibberellin signaling in plant cold stress responses

PK1.5 Stefanie Tischer, Christian Wunschel and Erwin Grill Laboratory (Botany, Technische Universität München)

The family of RCAR ABA receptors

PK1.6 Maren Livaja, Yu Wang, Grit Haseneyer, Silke Wieckhorst, Christina Kreim, Michael Seidel, Volker Hahn, Steven J. Knapp, Stefan Taudien, Chris-Carolin Schön, and Eva Bauer (Plant Breeding, Technische Universität München)

SNP marker enrichment in a specific genomic region of sunflower using bulked segregant transcriptome analysis.

PK1.7 M. M. Martis, R. Zhou, G. Haseneyer, T. Schmutzer, J. Vrana, H. Simkova, S. König, K. G. Kugler, U. Scholz, B. Hackauf, V. Korzun, J. Dolezel, E. Bauer, K. Mayer, and N. Stein (MIPS/IBIS, Helmholtz Center Munich, Germany)

The genome structure of rye and its multiple parents

PK1.8 Claudia Gehring and Kay Schneitz (Plant Developmental Biology, Technische Universität München)

The putative β -1,3 glucanase ZERZAUST localizes to plasmodesmata and participates in signal transduction mediated by the RLK STRUBBELIG

PK1.9 Melina Pepels and Pascal Falter-Braun (Plant Systems Biology, Technische Universität München, Germany)

Phytohormones in crosstalk

PK1.10 Stefan Altmann and Pascal Falter-Braun (Plant Systems Biology, Technische Universität München, Germany)

Natural variation in interactome networks

New insights to increased crop yield: Focusing on the importance of the microbiome

D. Ry Wagner

Agrinos, LLC, Lysaker, Norway

In the past few years the development of new detection methods – especially high-throughput DNA and RNA sequencing – has enabled determining the precise composition of complex microbial communities, including those organisms that cannot be cultured. Based on these data there has been a rapid advance in our understanding of the critical interactions between the microbial ecosystems that coexist with plants and animals. Through these analyses we are gaining a deeper understanding of long recognized and essential role of a plant's immediate biological ecosystem in enhancing plant nutrition and productivity. Much of this knowledge mirrors a similar new-found appreciation and understanding of the essential role of the microbiome in animal growth and development. This presentation will highlight some of the common insights on the role of the microbiome in both plant and animal development and some specific applications of this understanding for improving crop yield under field conditions.

Amino acid supply of sink tissues

Ulrich Z. Hammes

Cell Biology and Plant Biochemistry, University of Regensburg, Germany

In vascular land plants assimilates are transported from the site of their synthesis, the source tissues, to the sites of demand, the sink tissues. In the case of amino acids the long distance transport occurs in both the phloem and the xylem and the cycling of amino acids between source and sink tissues in both vasculature types is thought to contribute to the signaling of nutritional status within the plant. A number of transporters mediating the uptake of amino acids were described. To date little is known about the transporter that mediate the release of amino acids from cells into the apoplast. Recently, a family of transporters were identified that may play a role of in this process. We characterized a subset of transporters from this family in *Arabidopsis* whose expression is induced in response to plant parasitic root-knot nematodes and *Verticillium longisporum*, a soil-borne fungal pathogen that resides in the vasculature. The transporters are also expressed during seed maturation. We were able to identify transporters that are expressed specifically in the unloading domain of the vasculature at the chalazal pole, the outer integuments or the inner integuments. The specific expression indicates an important role in assimilate delivery to the developing embryo. Characterization of the transporters in *Saccharomyces cerevisiae* and *Xenopus laevis* demonstrated that the transporters are able to take up and to release amino acids from cells. The expression pattern and the physiological properties of these transporters are consistent with a role in amino acid supply of sink tissues.

Signal transduction in plant root symbiosis

Martin Parniske

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We are interested in unraveling the molecular mechanisms involved in the intracellular accommodation of symbiotic microorganisms by plants. Legumes form symbiosis with phosphate-acquiring arbuscular mycorrhiza fungi and nitrogen-fixing rhizobia. Forward genetics has identified a series of plant genes required for early developmental stages of both symbioses. The predicted protein products of these 'common symbiosis genes' include a receptor-like kinase, nuclear localized ion channels and components of the NUP84 sub-complex of the nuclear pore. These components act upstream of symbiosis-induced calcium spiking, which is likely to be decoded by a complex formed by a calcium- and calmodulin-dependent protein kinase and CYCLOPS, a nuclear protein with a coiled-coil domain. Recent progress in analyzing the function of individual symbiosis signaling components at the mechanistic level will be presented.

INHOSPITABLE is required for early steps of arbuscular mycorrhiza development

Caroline Gutjahr

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Our research focusses on elucidating plant molecular mechanisms that govern arbuscular mycorrhiza development. Arbuscular mycorrhiza (AM) is an ancient symbiosis between most land plants and glomeromycotan fungi that is based on the mutual exchange of nutrients between the two partners. For symbiosis establishment, AM fungi colonize the interior of the root, which involves dramatic re-differentiation of plant cells and their subsequent penetration by fungal hyphae. Colonization is preceded by an exchange of diffusible signals between root and fungus and fungal docking to the root surface via a differentiated hyphal structure, the hyphopodium. Plant factors that regulate hyphopodium formation, remain largely unknown. We identified a rice mutant that does not support hyphopodium formation and is therefore not colonized. The AM phenotype is due to deletion of the *Inhospitable* gene. Current work is characterizing the role of the INHOSPITABLE protein in AM symbiosis to increase our understanding of plant control over hyphopodium development.

Bacteria trigger systemic acquired resistance in the cereal crop barley

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Systemic acquired resistance (SAR) is an inducible, long-lasting, broad spectrum immune response that is well-characterized in dicotyledonous plants. By contrast, relatively little is known about systemic immune responses in monocotyledonous plants. Therefore, we investigated the possibility to induce systemic resistance in barley in order to establish a monocotyledonous systemic resistance pathosystem to test possible protection of cereals via SAR-like mechanisms. Infection of the first leaf of 4-week-old barley plants with either *P. syringae* pathovar *japonica* or *Xanthomonas translucens* significantly enhanced resistance in the systemic tissue against *X. translucens*. *P. syringae* growth was restricted in the infected leaf and caused numerous brown spots reminiscent of hypersensitive response lesions. *X. translucens* seemed virulent, causing spreading lesions, severe yellowing and eventually death. In contrast to SAR in dicotyledonous plants, SAR in barley does not appear to depend on salicylic acid (SA). The SA analog BTH did not trigger systemic resistance. In addition, systemic resistance was normal in barley plants silenced for the expression of *HvNPR1*, which in dicotyledonous plants encodes a master regulator of SA signalling. Subsequently, we performed RNAseq and microarray analyses of the local infected and systemic tissue to investigate which genes are induced and/or repressed during systemic resistance induction in barley. Results reveal that systemic resistance likely is primarily primed and not constitutively activated. Finally, we have established a mechanistic link between systemic resistance in barley and the defense hormone jasmonic acid (JA). Local treatment of barley with methyl-JA triggered systemic resistance. Taken together, we present the first evidence of systemic resistance in barley. This resistance can be triggered by a local pathogen infection or methyl-JA treatment and is most likely based on priming.

Breaking the DNA binding code of *Ralstonia solanacearum* TAL effectors provides new possibilities to generate plant resistance genes against bacterial wilt disease

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Ralstonia solanacearum, the causal agent of bacterial wilt disease, has a broad host range and infects many economically important crop and ornamental species. Genetically determined disease resistance is desirable to control this pathogen. However, till now no plant resistance (*R*) gene has been identified that provides control to a broad range of *R. solanacearum* strains in economically important plant host species.

Previously we studied the molecular basis of plant *R* genes that mediate recognition Transcription activator-like effectors (TALEs) from the bacterial pathogen *Xanthomonas*. TALEs from *Xanthomonas* act like plant transcription factors, bind to defined effector binding elements (EBE) in given plant promoters and activate the downstream host genes to promote disease. Structure-function studies of TALEs and matching EBEs uncovered that these effectors bind to DNA via tandemly arranged 34-amino acid repeats. Each TALE repeat binds to one base in the cognate EBE and amino acid 13 in each TALE repeat determines base specificity. Our insights into TALE function and natural TALE-specific plant *R* genes enables us by now to generate sophisticated synthetic *R* genes that mediate recognition of multiple TALEs.

The vast majority of *R. solanacearum* strains contain TALE-like proteins and thus these effector proteins provide an ideal target to deduce corresponding plant *R* genes. Our studies on TALE-like from *R. solanacearum* (RipTALs) revealed that RipTALs, like *Xanthomonas* TALEs bind to DNA via a central repeat domain. As in TALEs, RipTAL base specificity is determined by repeat residue 13. Yet in contrast to *Xanthomonas* TALEs, that bind preferentially to EBEs that are preceded by a 5' thymine, RipTALs have a strong preference for a 5' guanine. The deciphered RipTAL code now provides a basis to generate synthetic RipTAL-specific *R* genes. Recent progress on these RipTAL-specific *R* genes will be presented.

Pecha kucha - Session 2 (Inês Barbosa, Technische Universität München)

PK2.1 Astrid Fastner, Benedikt Müller, and Ulrich Z. Hammes (Cell Biology and Plant Biochemistry, University Regensburg, Germany)

The BAFs: Bidirectional amino acid transporters with a role in phloem unloading in sink tissues

PK2.2 Priya Pimprikar, Caroline Gutjahr, and Martin Parniske (Institute of Genetics, LMU Munich, Germany)

***red*, a *Lotus japonicus* mutant perturbed in arbuscule-related gene expression and development**

PK2.3 Andreas Keymer, Caroline Gutjahr, Aline Banhara, Vera Wewer, Simone Hardel, Edda von Roepenack-Lahaye, Peter Dörmann, and Martin Parniske (Institute of Genetics, LMU Munich, Germany)

DISORGANIZED ARBUSCULES is required for arbuscule branching

PK2.4 Samy Carbonnel, Maximilian Griesmann, Trevor Wang, and Caroline Gutjahr (Institute of Genetics, LMU Munich, Germany)

Unraveling the function of INHOSPITABLE in arbuscular mycorrhiza symbiosis of *Lotus japonicus*.

PK2.5 Elisabeth Pabst, Heiko Breitenbach, Finni Wittek, Jane E. Parker, and A. Corina Vlot-Schuster (Helmholtz Zentrum München, München-Neuherberg, Germany)

The proteome of systemic acquired resistance: identification of proteins involved in the regulation of systemic immunity

PK2.6 Marlies Bichlmeier, Finni Wittek, Thomas Hoffmann, Wilfried Schwab, and A. Corina Vlot-Schuster (Helmholtz Zentrum München, München-Neuherberg, Germany)

The metabolome of systemic acquired resistance: identification of metabolites that trigger systemic immunity

PK2.7 Niklas Schandry and Thomas Lahaye (Institute of Genetics, LMU Munich, Germany)

A sophisticated promoter trap mediates recognition of phylogenetically diverse *Ralstonia solanacearum* strains

PK2.8 Katrin Franz and Wilfried Schwab (Biotechnology of Natural Products, Technische Universität München)

Validation of FaAP as interacting partner of Fra a proteins

PK2.9 Fatma Besbes and Wilfried Schwab (Biotechnology of Natural Products, Technische Universität München)

The Fra a gene family in *Fragaria x ananassa*

PK2.10 Friedericke Bönisch and Wilfried Schwab (Biotechnology of Natural Products, Technische Universität München)

Functional characterization of UDP-glucose:monoterpenol glucosyltransferase genes from *Vitis vinifera*

PK2.11 Neele Wendler, Martin Mascher, Axel Himmelbach, Uwe Scholz, Christiane Nöh, Brigitte Ruge-Wehling, Nils Stein (Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben)

Utilizing next-generation-sequencing to unlock barley's secondary gene pool

PK2.12 Lisa Kappel, Karin Zwerger, Nikola Winter, Wenwen Huang and **Tobias Sieberer** (Plant Growth Regulation, Technische Universität München)

Ramostatin, a novel small molecule enhancer of polar auxin transport that inhibits shoot branching

Intracellular machineries for movement of endoplasmic reticulum and nucleus in *Arabidopsis thaliana*

Ikuko Hara-Nishimura, Kentaro Tamura and Haruko Ueda

Department of Botany, Graduate School of Science, Kyoto University, Japan

Plants exhibit an ultimate case of the intracellular motility involving rapid organelle trafficking and continuous streaming of the endoplasmic reticulum (ER). A detailed velocity distribution map for the GFP-labeled ER shows that the ER dynamics is driven primarily by the ER-associated myosin XI-K, a member of a plant-specific myosin class XI. The myosin XI deficiency affects organization of the ER network and orientation of the actin filament bundles. We suggest a model whereby dynamic three-way interactions between ER, F-actin, and myosins determine the architecture and movement patterns of the ER strands, and cause cytosol hauling traditionally defined as cytoplasmic streaming (1). On the other hand, a striking feature of plant cells is dark-induced positioning of the cell nucleus in mesophyll cells. The nuclear movement is also driven by actin/myosin machinery, although the motility machinery is different from that for the ER movement. We isolated an *Arabidopsis thaliana* mutant, in which nuclear movement was impaired and the nuclear envelope was abnormally invaginated. Characterization of the mutant provides a new type of nucleocytoplasmic linker consisting of a myosin XI-i motor and nuclear membrane proteins (2). This machinery moves along an actin filament cytoskeleton, in contrast to animal nuclei movement by kinesin and dynein motor proteins along the microtubule cytoskeleton. The unique nucleocytoplasmic linkage in plants might enable nuclear positioning in response to environmental stimuli.

1. Ueda et al., Proc Natl. Acad. Sci. (2010) 107, 6894-9.

2. Tamura et al., Current Biol. (2013) published online

RAC/ROP GTPase signalling in microtubule organization and susceptibility of barley to powdery mildew

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Little is known about the nature and function of host factors involved in susceptibility to plant diseases. Host factors may be involved particularly in cellular reprogramming for accommodation of biotrophic fungal infection structures in intact plant cells. The barley RAC/ROP monomeric G-protein RACB is required for full susceptibility to the powdery mildew fungus, *Blumeria graminis* f.sp. *hordei*, and it is involved in cell polarity and cytoskeleton organization. Under fungal attack, cortical microtubules strongly polarize to sites of successful defence at cell wall papillae. In contrast, microtubules locally loosen when the fungus succeeds in penetration. We identified a novel microtubule associated RAC/ROP-GTPASE ACTIVATING PROTEIN (MAGAP1) and a ROP binding receptor-like cytoplasmic kinase (RBK1) interacting with RACB in yeast and *in planta*. Fluorescent MAGAP1 decorated cortical microtubules and can be recruited by constitutively activated CA RACB to the plasma membrane. Reverse genetic experiments suggest that both MAGAP1 and RBK1 are involved in negative feedback on RACB function in loosening microtubule arrays. CA RACB supports fungal entry most likely by destabilizing microtubules. ROP INTERACTIVE PARTNER 3/MICROTUBULE DEPLETION DOMAIN1 (RIP3/MIDD1) can interact with RACB-like proteins and KINESIN13A in Arabidopsis. A complex of RIP3/MIDD1 and KINESIN13A destabilized microtubules in Arabidopsis as well as in barley providing a potential mechanistic link between RAC/ROPs and microtubule loosening.

These results add to our understanding of how intact plant cells accommodate biotrophic infection structures and establish RACB and associated proteins as key players in re-organization of microtubules under fungal attack. The possible activation of RACB by a novel peptide effector from *Blumeria graminis* is discussed.

The role of KDEL-tailed cysteine endopeptidases in programmed cell death of plants

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Programmed cell death (PCD) is a genetically determined process in all multicellular organisms. Plant PCD is effected by a unique group of papain-type cysteine endopeptidases (CysEP) with a C-terminal KDEL endoplasmic reticulum (ER) retention signal (KDEL CysEP). KDEL CysEPs are stored as pro-enzymes in ER-derived endomembrane compartments and are released as mature CysEPs in the final stages of organelle disintegration. KDEL CysEPs accept a wide variety of amino acids at the active site, including the glycosylated hydroxyprolines of the extensins that form the basic scaffold of the cell wall. In *Arabidopsis*, three KDEL CysEPs (*AtCEP1*, *AtCEP2*, and *AtCEP3*) are expressed. Cell- and tissue-specific activities of these three genes suggest that KDEL CysEPs participate in the collapse of tissues in the final stage of PCD and in developmental tissue remodelling.

We have constructed the fluorescing reporter proteins $P_{CEP1}::pre-pro-3xHA-EGFP-CEP1-KDEL$ and $P_{CEP2}::pre-pro-3xHA-mCherry-CEP2-KDEL$ in transgenic *Arabidopsis* in order to characterize their spatio-temporal appearance in the course of PCD. The non-functional reporter proteins without the protease subunit $P_{CEP1}::pre-pro-3xHA-EGFP-KDEL$ and $P_{CEP2}::pre-pro-3xHA-mCherry-CEP2-KDEL$ were also transformed into the knock-out *atcep1* and *atcep2* lines, respectively, in order to investigate the mutant phenotype.

Here, we describe the involvement of *AtCEP2* in root development. mCherry-*AtCEP2* was detected in the epidermal layers of leaves, hypocotyl and roots of young seedlings, where it was predominantly expressed in elongation zone and root cap.

Furthermore, we observed that *AtCEP1* is expressed in response to biotic stress stimuli. *atcep1* knockout mutants showed enhanced susceptibility to powdery mildew caused by the biotrophic ascomycete *Erysiphe cruciferarum*. $P_{CEP1}::pre-pro-3xHA-EGFP-AtCEP1-KDEL$ rescued the powdery mildew phenotype demonstrating the functionality of the fusion protein. The spatio-temporal gene expression and protein appearance of tagged versions of *AtCEP1* during fungal infection of leaves suggested a function in controlling late stages of compatible interaction.

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

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Intracellular trafficking is essential for various aspects of plant growth and development. Our group is interested in the regulation of membrane trafficking by posttranslational modification mediated by ubiquitin. Ubiquitination of substrates usually triggers the endocytosis of the substrate protein, upon which it is transported to the vacuole for degradation by vacuolar proteases.

Ubiquitination is a strictly regulated process in which substrate proteins are ubiquitinated by specific ubiquitinating enzymes. This reaction can be reversed by deubiquitinating enzymes (DUBs). Ubiquitinating enzymes and DUBs can thus both contribute to the stability of a target protein. Associated Molecule with the SH3 domain of STAM (AMSH) is a metalloprotease that belongs to the MPN+ domain containing DUB family.

The *Arabidopsis* genome encodes three *AMSH* genes. We have previously shown that AMSH3 is an essential DUB in *Arabidopsis* that interacts with core subunits of the Endosomal Sorting Complex Required for Transport (ESCRT)-III. ESCRT-III is required in late stages of endocytosis for membrane scission during cargo sequestration into intraluminal vesicles of MVBs. AMSH1, an AMSH3-related DUB, also interacts ESCRT-III and thus might be involved in intracellular trafficking.

Our physiological and cell biological analyses of *Arabidopsis amsh* mutants revealed a function of AMSH proteins together with ESCRT-III not only in endocytosis but also in autophagic degradation and proper autophagic response. We are currently conducting further analyses with the aim to understand the molecular framework supporting *Arabidopsis* AMSH function.

Interplay between phosphorylation and SUMOylation events determines CESTA protein fate in brassinosteroid signaling

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Brassinosteroids (BRs) are steroid hormones that control fundamental aspects of plant growth and development. The BRs signal through the inhibition of glycogen-synthase-like kinases 3, which, as previous models have proposed, phosphorylate transcription factors of the BES1/BZR1 subfamily to alter their protein stability and DNA binding activities. Here we present evidence for a novel molecular mode of BR signaling. We show that BRs regulate the activity of CESTA (CES), a bHLH transcription factor that controls BR responsive gene expression. In response to BRs CES nuclear localization reorganizes from a diffuse localization to a speckled pattern. We provide evidence that this BR-induced nuclear body formation is regulated by an antagonistic cross-talk of phosphorylation and SUMOylation events, which in addition to nuclear distribution also control CES protein abundance and transcriptional activity. We present a model in which phosphorylation, mediated by distinct classes of kinases at different sites, controls the SUMOylation status of CES, implicating SUMOylation in the control of BR responses and providing a mechanistic explanation for nuclear body formation in plants.

Pecha kucha - Session 3 (Andreas Keymer, LMU Munich)

PK3.1 Björn Scheler, Lars Voll, Timo Engelsdorf, Ralph Hückelhoven (Phytopathology, Technische Universität München)

Investigations on HvRACB-dependent cell wall modifications in barley with reduced susceptibility to powdery mildew

PK3.2 Vera Schnepf, Corina Vlot, Ralph Hückelhoven (Phytopathology, Technische Universität München)

Barley HvRACB influences pathogenesis-related transcriptional patterns to powdery mildew

PK3.3 Mathias Nottensteiner, Ruth Eichmann, Ralph Hückelhoven (Phytopathology, Technische Universität München)

Barley HvRACB is a potential target of an atypical effector of *Blumeria graminis*

PK3.4 Timo Höwing and Christine Gietl (Botany, Technische Universität München, Germany)

The role of KDEL-tailed cysteine endopeptidases in programmed cell death of plants (Poster withdrawn)

PK3.5 Cornelia Kolb and Erika Isono (Plant Systems Biology, Technische Universität München, Germany)

Characterization of a vacuolar fusion defective mutant in *Arabidopsis thaliana*

PK3.6 Julia Mergner and Claus Schwechheimer (Plant Systems Biology, Technische Universität München, Germany)

Identification of neddylation substrates in *Arabidopsis den1* mutants

PK3.7 Linlin Zheng, Monika Frey and Alfons Gierl (Genetics, Technische Universität München)

DNA elements required for late *Bx*-gene expression in the maize line Mo17.

PK3.8 Stefan Lenk, Monika Frey and Alfons Gierl (Genetics, Technische Universität München)

Transgenic expression of the maize genes Bx1 and Bx2 in *Arabidopsis thaliana*: Impact on metabolism and biotic interaction.

PK3.9 Doreen Schiller and Wilfried Schwab (Biotechnology of Natural Products, Technische Universität München)

Contribution of the lipoxygenase gene family to flavor formation in apple (*Malus domestica*) fruit

PK3.10 S. Mucha, T. Müller, A. Chapman, D. Walther, C. Böttcher, and E. Glawischnig (Genetics, Technische Universität München)

Metabolic engineering and evolutionary analysis of phytoalexin response in *Brassicaceae* including *Thellungiella/Eutrema* as a new model system

PK3.11 Mark Zander, Corinna Thurow, Christiane Gatz (Albrecht-von-Haller Institute for Plant Sciences, Göttingen University, Göttingen)

Suppression of the ethylene defense response by salicylic acid is mediated by TGA transcription factors

Enhancing crop productivity through manipulation of reproductive biology

Marc C. Albertsen, Mark Cigan, Tim Fox, Howard Hershey, Mary Trimnell, and Yongzong Wu

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Development of hybrids has not been fully enabled in crops where current methods of hybrid production are either inefficient or limit the full range of germplasm utilization. We have taken a directed molecular genetic approach, using maize as a model system, to develop several genetic hybridization platforms that not only produce male-sterile plants, but that enable these male-sterile plants to produce nearly 100% male-sterile progeny upon increase. This includes both recessive and dominant-acting systems that can be applied not only to maize but to other crops where the reproductive biology of that crop has been an impediment to wider utilization of hybrids in those crops. One of these is a process designated as SPT (Seed Production Technology). It utilizes a recessive mutation in a sporophytic gene required for male fertility, creating female parent lines that are male sterile when the mutant allele is homozygous, yet enabling the production of nearly 100% male sterile, non-transgenic progeny. Full male fertility is restored in hybrid progenies upon pollination of the male-sterile female parent plants with pollen from any male parent carrying a wild-type allele of the mutant male-sterility gene. The SPT process offers a reliable, cost-effective method to propagate pure populations of homozygous recessive male-steriles that are non-transgenic for the SPT process. This process is well-suited to crops that are molecularly characterized and can be a first step in developing or expanding the use of hybrids in those crops. For crops that are not as well-characterized, other dominant systems have been developed. Advantages and disadvantages of both types of systems in forming the basis of producing new crop hybrids will be discussed.

Signaling during pollen tube perception

Mayada Woriedh ¹, Philipp Denninger ¹, Andrea Bleckmann ¹, Rainer Merkl ², Christine Drübert ³, Guido Grossmann ⁴, Dirk Becker ³, and **Thomas Dresselhaus** ¹

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Recent years have shown that proper cross-talk among gametophytic cells represents a key to reproductive success in flowering plants. In addition to gametophytic interactions between pollen tube and embryo sac cells, cell-cell-communication occurs inside both gametophytes as well as between gametic cells during pollen tube burst. Here we will focus on communication during pollen tube perception and discuss two classes of small cysteine-rich proteins (CRPs) that are involved to induce pollen tube burst. Additionally, we will report on possible roles of calcium signaling during this process. The defensin-like (DEFL) small protein ZmES4 (*Zea mays* Embryo Sac4) was previously shown to induce pollen tube burst via opening of the potassium channel KZM1 (Amien et al. 2010, PLoS Biol.). Here we show that other members of the ZmES family are also capable to enhance the open probability of potassium channels at physiological membrane potentials. Mutated proteins and short peptides derived from various domains of ZmES4 were used to map active sites and channel interaction domains. A second class of small CRPs secreted from the embryo sac encode PME inhibitors (PMEI) that destabilize the pollen tube wall after external application suggesting that they work in concert with DEFL-like toxins to induce pollen tube burst (Woriedh et al. 2013, Plant Reprod.). Finally, we have studied the role of calcium during pollen tube perception by monitoring its dynamics in the synergids, egg and central cell by using a novel troponin-based biosensor.

Posttranslational regulation of sperm-activating EC1 proteins

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Sexually reproducing organisms require an orchestrated communication between the two gametes of opposite sex to accomplish cell-cell fusion. In vertebrate and non-vertebrate species egg-sperm interactions mainly depend on cell surface proteins. Flowering plants have evolved the unique reproductive strategy of double fertilization, where two distinct gamete fusion events take place in a coordinated manner (1). Two immotile sperm cells are delivered by a pollen tube into the female gametophyte (embryo sac) that harbors two dimorphic female gametes. One sperm fuses with the egg cell, giving rise to the embryo, whereas the second sperm cell fuses with the central cell to develop the triploid endosperm. Little is known about cell surface proteins involved in flowering plant gamete interactions.

Recently, we reported about the identification of a small family of egg cell-secreted cysteine-rich proteins (CRPs), which turned out to be essential signaling molecules for successful double fertilization in *Arabidopsis thaliana* (2). EC1 proteins accumulate within the unfertilized egg cell and become specifically secreted upon sperm arrival. Bioassays using synthetic EC1 peptides suggest that this protein family is involved in activating the sperm endomembrane system, leading to surface exposure of membrane-active fusion-essential sperm proteins.

Notably, we found the EC1 proteins to be highly unstable molecules. The expression of EC1 proteins is tightly regulated, not only on the transcriptional but also on the posttranslational level. I will report about our recent results regarding EC1 stability, EC1 function, and the identification of an EC1-interacting protein involved in post-translational protein modification.

(1) Berger, F., Hamamura, Y., Ingouff, M., Higashiyama, T. (2008) Double fertilization - caught in the act. *Trends Plant Sci.* 13, 437

(2) Sprunck, S., Rademacher, S., Vogler, F., Gheyselinck, J., Grossniklaus, U., Dresselhaus, T. (2012) Egg cell-secreted EC1 triggers sperm cell activation during double fertilization. *Science* 338, 1093-1097

Live-cell study in combination with synthetic chemistry: pollen tube guidance as a model

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Sexual plant reproduction for yields is difficult to study in the living material due to an embedded structure of female reproductive cells. We have been working on pollen tube guidance, double fertilization, and early embryogenesis in the living material by using two model plant species, *Torenia fournieri* and *Arabidopsis thaliana*. Defensing-like peptide LUREs are pollen tube attractants of these species working in a short distance (a few hundred micrometers), which are secreted by two synergid cells on the side of the egg cell (Higashiyama et al., 2001, Science; Okuda et al., 2009, Nature; Takeuchi and Higashiyama, 2012, PLoS Biol.). To understand the molecular mechanism of pollen tube guidance, we have been taking two approaches of live-cell study (for review, Kurihara et al., 2013, Cell Growth Differ.). The first approach is to use precisely defined *in vitro* system, including development of various microfluidics devices by our engineering team (e.g., Horade et al., 2012, Proc. MicroTAS). Recent *in vitro* studies lead to discovery of novel intercellular signaling molecules involved in competency control of pollen tubes and long-distance attraction (a few millimeters). The second approach is based on *in vivo* imaging. We have shown that pollen tube guidance is intimately related with double fertilization (Hamamura et al., 2011, Curr. Biol.; Kasahara et al., 2012, Curr. Biol.; Maruyama et al., 2013, Dev. Cell). In this symposium, full-scale collaboration between chemistry and biology in our new institute will also be introduced.

A cell biological survey of the heterogeneous membrane micro-domain landscape of plant cells

Thomas Ott

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Increasing evidence has demonstrated that physical interactions between plasma membrane resident receptors and other signaling proteins often occur in distinct membrane domains. In human cells, membrane-bound molecular scaffold proteins mediate the assembly of such micro-domains. They interact with a variety of signaling proteins to form super-complexes and structurally define domain patterning. Although plants have massively diversified their signaling protein repertoire, similar evidence has never been presented. To unravel the diversity of the membrane domain landscape of living cells in intact multicellular tissues we cloned and expressed nineteen different putative molecular scaffold proteins from *Arabidopsis thaliana* that belong to the remorin and flotillin protein families. All of them associate with the cytosolic leaflet of the plasma membrane and segregate into distinct and immobile membrane domains. Systematic co-localization experiments allowed lateral discrimination of a variety of distinct domain patterns. While most of these micro-domains do not show any lateral mobility, we demonstrate efficient recruitment of freely diffusing proteins into and release from these sites. Furthermore we can show that physical interactions between receptors and molecular scaffolds occur in distinct micro-domains. This work provides a global view on the cell biology of membrane domains and their spatial diversity in plant cells. These novel set of specific domain marker proteins will allow to study dynamics and lateral segregation of signaling complexes at the plasma membrane in response to biotic, abiotic and developmental cues.

Pecha kucha - Session 4 (Marlies Bichlmeier, HMGU München-Neuherberg)

PK4.1 Mayada Woriedh, Sapna Sharma, Klaus Mayer, and Thomas Dresselhaus (Cell Biology and Plant Biochemistry, University of Regensburg, Germany)

Genome-wide annotation of the maize transcriptome revealed thousands of novel genes including various subclasses of CRPs

PK4.2 Maria Enghart and Stefanie Sprunck (Cell Biology and Plant Biochemistry, University of Regensburg, Germany)

Gamete interaction and mutual gamete activation in *Arabidopsis thaliana*

PK4.3 Thomas Hackenberg and Stefanie Sprunck (Cell Biology and Plant Biochemistry, University of Regensburg, Germany)

Cell surface proteins mediating gamete interaction in *Arabidopsis*

PK4.4 Frank Vogler and Stefanie Sprunck (Cell Biology and Plant Biochemistry, University of Regensburg, Germany)

Key factors promoting polar tip growth of *Arabidopsis* pollen tubes

PK4.5 Macarena Marín, Veronika Thallmair, Christoph Strotbek, Wolfgang Frank, and Thomas Ott (Institute of Genetics, LMU Munich, Germany)

Comparative genetics of remorin proteins

PK4.6 Corinna A. Hofer, Iris K. Jarsch and Thomas Ott (Institute of Genetics, LMU Munich, Germany)

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

PK4.7 Sebastian S. A. Konrad, Claudia Popp, Thomas F. Stratil and Thomas Ott (Institute of Genetics, LMU Munich, Germany)

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

PK4.8 Matthias Jost, Martin Mascher, Axel Himmelbach, Uwe Scholz, Arnis Druka, Robbie Waugh, Shin Taketa, and Nils Stein (Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben)

Cloning of the gene *Laxatum* (*lax.a*) - prospects from an improving barley genomics infrastructure

PK4.9 Mingjiu Li, Martin Mascher, Stefan Hiekel, Matthias Jost, Axel Himmelbach, Sebastian Beier, Viktor Korzun, Jochen Kumlehn, Thomas Börner and Nils Stein (Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben)

Molecular cloning and functional characterization of the gene *albostrians* in barley – towards a better understanding of chloroplast development

PK4.10 Michaela Matthes, Miriam Luichtl, Birgit S. Fiesselmann, Xiaomeng Yang, Otilie Peis, Andrä Brunner, and Ramon A. Torres-Ruiz (Genetics, Technische Universität München)

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

PK4.11 Benjamin Weller and Claus Schwechheimer (Plant Systems Biology, Technische Universität München)

Phosphorylation of PIN1 by the D6 PROTEIN KINASES

PK4.12 Inês Barbosa and Claus Schwechheimer (Plant Systems Biology, Technische Universität München)

Polar targeting of the AGCVIII kinase D6PK and its implications on polar auxin transport

The genetic architecture of maize domestication: Low hanging fruit and dark matter

John Doebley

Genetics Department, University of Wisconsin-Madison, USA

The domestication of maize from a wild Mexican grass called teosinte occurred about 9,000 years ago and resulted in dramatic changes in plant morphology. The genetic changes that underlie maize domestication have been investigated using quantitative trait locus (QTL) mapping, QTL cloning, genome-wide selection scans, and genome-wide scans for altered gene expression. QTL analyses suggest that some morphological traits are governed by relatively large numbers of genes (20 or more), but that other traits have relatively simple inheritance involving a single QTL of large effect plus a few smaller effect QTL. We have identified and characterized QTL (genes) with large effects on some domestication traits. First, *teosinte branched* (*tb1*) is largely responsible for the difference between the long branches of teosinte versus the short branches of maize. *tb1* encodes a transcriptional regulator that functions as a repressor of branch elongation. Gene expression analysis indicates that the transcript of the teosinte allele of *tb1* accumulates at about half the level of the maize allele. Fine-mapping experiments show that the differences in phenotype and gene expression are controlled in part by an upstream transposon insertion that acts as an enhancer of gene expression. Second, *teosinte glume architecture* (*tga1*) is largely responsible for the formation of a casing that surrounds teosinte seeds but is lacking in maize. *tga1* also encodes a transcriptional regulator, however in this case a single amino acid change represents the functional difference between maize and teosinte. This single amino acid change appears to convert the maize allele into a transcriptional repressor of target genes. Third, *grassy tillers* (*gt1*) contributes to differences in plant architecture and encodes an HD-ZIP transcription factor. A *cis*-regulatory change at *gt1* alters its tissue specific expression pattern. While QTL studies enabled the identification and characterization of a few domestication genes for morphological traits, genomic scans have identified hundreds of genes that show evidence for selection during domestication or differential expression between maize and teosinte, suggesting that selection during domestication may have targeted a broad array of genes controlling unknown traits.

Sequencing diverse rice germplasms for GWAS of complex traits and rice domestication study

Bin Han

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The rice domestication and breeding have a profound influence on the genetic diversity present in modern rice cultivars. Identification of genetic basis of phenotypic variations and domestication processes would provide new opportunities for rice improvement. Our major research interest is to develop new genomic approaches for tapping these resources to provide rice researchers a powerful new resource for rice improvement. Work in our lab has focused on accessing genome-scale sequence variation in cultivated rice varieties (*Oryza sativa*) and closely-related wild rice accessions (*Oryza rufipogon*) using re-sequencing.

In our study, association genetic approaches have been developed and used to study flowering time and yield traits in a worldwide collection of rice germplasm. We also detected genome sequence variations in a large population of rice accessions including wild rice species and cultivated rice varieties and used these data to reveal the origins of cultivated rice and to investigate rice domestication processes. By integrating the analysis of genome-wide patterns and population genetics study, we revealed that the cultivated rice was first domesticated from a specific population of *O. rufipogon* in southern China. For defining domestication processes, we also cloned and functionally analyzed two rice domestication-related genes such as the *Bh4* gene controlling a transition from black to straw-white seed hull, and the *An-1* gene controlling awn development during the rice domestication. Our study provides an important resource for rice breeding and an effective genomics approach for crop domestication research.

Genome-enabled prediction of complex phenotypes – advances in quantitative genetics

Chris-Carolin Schön

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Agricultural genetics is currently revolutionized by technological developments in genomic research. High-throughput genotyping technology delivers hundreds of thousands of single nucleotide polymorphism markers and has become available for many crops and livestock species. The technological feasibility of obtaining full genome sequences at reasonable costs for a large number of individuals is within striking distance. Thus, genetic analysis of quantitatively inherited traits and prediction of the genetic predisposition of individuals based on molecular data are rapidly evolving fields of research in agricultural genetics.

This talk presents quantitative genetic approaches that make it possible to extract knowledge on the genetic value of individuals from high-dimensional genomic data and predict the genetic makeup of individual offspring. Statistical methods for prediction of genetic values and phenotypes from genome-wide molecular marker data will be introduced and challenges arising from the large number of predictors and their high degree of collinearity will be addressed. Whole-genome based approaches will be compared to marker-assisted prediction based on individual genomic regions identified from quantitative trait locus mapping and the role of variable selection for improving accuracies of prediction will be discussed.

The efficiency of genome-enabled prediction will be demonstrated with experimental studies on grain yield and insect resistance in maize (*Zea mays* L.). Estimates of prediction accuracies achieved in these studies are encouraging with respect to the usefulness of genome-enabled prediction in practical breeding programs. Thus, optimum scenarios for exploiting knowledge from high-dimensional molecular data in breeding schemes will be discussed.

FT control of potato storage organ formation: new prospective to the selection of heat-stress cultivars

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Potato is the third largest global food crop after wheat and rice. This crop is cultivated for its underground storage organs or tubers, which are very rich in starch and vitamin C. Referred to dry weight, protein content of potato tubers is similar to that of cereals. Amino acid composition, however, is much better balanced to the human dietary needs than in cereal grains. Easy cultivation and elevated yields per hectare made FAO designate this crop as strategic to the eradication of poverty.

Potato tubers differentiate from underground stems or stolons. In nature, these organs are formed as winter approaches and remain dormant in soil until next spring, in which they germinate and generate a new plant. Plants use seasonal changes in day length and temperature as the informational cues to trigger formation of these organs. Day length is perceived by the leaves. When nights are longer than a minimal threshold (critical photoperiod), a mobile signal or “tuberigen” is produced in the leaves and transported via the phloem to the underground stolons to initiate tuber formation. We have shown that this mobile signal is encoded by a member of the *FLOWERING LOCUS T (FT)* gene family, the *SP6A* gene. In Solanaceae this gene family has undergone preferential expansion, with 5 members identified in the potato genome. Interestingly, the transcriptional factor CONSTANS does not regulate expression of the *SP6A* mobile tuberization signal, but activates an additional member of the gene family, the *SP5G* gene. *SP6A* is only up-regulated in phloem cells when the negative *SP5G* regulator is not expressed. The nature of the factor responsible to activate *SP6A* expression in SDs is still unknown, although gene expression studies indicate that this factor is directly regulated by *SP5G*. Search for *SP5G* interactors is now underway to gain insights into this important regulator.

High night temperatures are inhibitory to tuber formation and this inhibition is correlated with a strong reduction in *SP6A* transcript levels. We have generated transgenic lines expressing the *SP6A* protein under control of a heat-shock inducible promoter and shown that activation of this gene compensates the inhibitory effects of elevated temperatures. These lines produce high yields even at temperatures higher than 30°C and are suitable to be cultivated in tropical areas. Collaboration with research Institutes in Africa and India will help to the selection of new varieties with stable *SP6A* gene expression at high temperatures. These should be suitable to cultivation in warm areas and contribute to assure food chain supply. Research in cassava will also help defining if similar regulatory pathways play a role in tuberous root formation, with these findings being made extensive to other tropical crops.

Navarro C. et al. (2011) Control of flowering and storage organ formation in potato by *FLOWERING LOCUS T*. *Nature* 478:119-22.
Rodríguez-Falcón M. et al. (2006) Seasonal control of tuberization in potato: conserved elements with the flowering response. *Annu Rev Plant Biol.* 57:151-80.

Where have all the plant phenotypes gone?

Dani Zamir

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Phenotyping is a rate-limiting activity in genomic research. Plant geneticists and breeders have generated over the past decade numerous Mendelian populations that segregate for genetically mapped quantitative trait loci (QTL). Information about the map positions of QTL is included in more than 5000 publications but only a small fraction of raw data finds its way into existing genomic databases. As opposed to sequence and expression data that need to be deposited in appropriate databases upon publication, the raw data of replicated phenotypic measurements are not deposited in any public repository and are usually lost. A major bioinformatic challenge facing the research community is to develop web-based resources to display the details of complex phenotypes to uncover hidden biological knowledge. Phenom Networks <<http://www.phenome-networks.com/>> provides such a platform where researchers can share data and compare their phenotypes and analyses to those that have already been deposited in the database to identify wider pleiotropic links. Uniting data from multiple syntenic crops on a common framework will enable the identification of common and unique bottlenecks for crop productivity and the formulation of rational strategies for genomic assisted breeding. The future sharing of phenomic data is the key for continuity of collaborative projects both in academic labs and the seed industry, and this vision will be demonstrated using results from our laboratory. What we eat are phenotypes - in view of the need to achieve global food security it is high time that we find efficient ways to link traits with genomes.

Zamir D (2013) Where Have All the Crop Phenotypes Gone? PLoS Biol 11(6): e1001595.

Hosting three genomes: the bread wheat genome and its transcriptional interplay during grain formation

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Bread wheat (*Triticum aestivum*) is one of the most important crops worldwide. Access to the hexaploid wheat genome is hampered by the enormous size, high repeat content and polyploidy. We developed approaches that seek to circumvent these limitations by making use of different complementary strategies that aim to detect, assemble and position genes along the chromosomes.

For the time being combined and complementary approaches to reduce economic and technological limitations are pragmatic solutions and allow to address genome biological questions and to start to address systems biology questions. Using the emerging genome sequence as an information backbone in depth transcriptional profiling of the developing grain in context of a hexaploid genome is undertaken and used to gain insights into the transcriptional coordination among three homeologous subgenomes.

We performed deep profiling of the global transcriptome of single endosperm cell types (starchy endosperm, aleurone and transfer cells, respectively) to test for the effects of polyploidy on gene expression of homeologous, duplicated genes in bread wheat. A total of 556mio RNA-seq read pairs (103Gb) were used and resulted in the identification of approx. 61,000 expressed high-confidence protein-coding wheat genes as well as 10,000's of novel alternative splicing variants. Expressed genes were subject to network-based co-expression analysis revealing significant cell-type and time point specific gene expression. None of the subgenomes dominates gene expression in any co-expression model, however considerable change in gene expression for more than 90% of strictly associated homeologous gene triplets was found. Extensive transition of homeologous gene expression between different modules indicates cell type specific expression, sub-functionalization of duplicated wheat genes as well as transcriptional silencing of one or two copies.

The presentation will aim to highlight progress in wheat genome sequencing and a systems biology use case in the multidimensional transcriptional analysis of the developing grain.

The application of molecular genetics for sustainable wheat production

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Our lab aims to understand the molecular mechanisms underlying important agronomic traits in wheat and, using this knowledge, to develop informed strategies to modify the crop's performance in the field. We are attempting to understand a series of traits that directly relate to yield and yield stability in farmer's field. These traits include yield per se as well as other yield-related traits such as grain size, grain filling duration, and pre-harvest sprouting. These are all multigenic traits which can have contrasting roles in affecting yield depending on the specific environment. The complexity of these traits is further compounded by the polyploid nature of wheat.

Through the use of near-isogenic lines across multiple environments we have recently validated a QTL affecting grain width, flowering time and final crop maturity. The region increases yield by an average of ~4.5%, equivalent to the gains made by an average UK breeder in 9 years. We will present our latest progress in unravelling these multiple effects using recombinant lines across the region. We will also discuss how understanding the mechanism underlying this QTL should enable us to exploit variation in the other homoeologous genomes and relate this to our current knowledge of grain size genes in rice. We will also outline our work on in silico TILLING in polyploid wheat and how this will enable use quickly validate candidate genes emerging from fine mapping projects. We hope this resource will enable more researchers to extend their work into these important crop species and fully exploit the (ongoing) release of wheat genome sequence.

From phenotypes to pathways: global exploration of cellular networks using yeast functional genomics

Brenda J. Andrews

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The entire landscape of eukaryotic genetic research has been transformed by our ability to rapidly sequence genomes – while we can now map genomes efficiently, we do not yet know how to interpret genome variation to predict inherited phenotypes. Emerging evidence suggests that we must account for genetic interactions in order to relate genotype to important phenotypes in any eukaryotic system. To systematically explore genetic interactions, our group developed a unique functional genomics platform called ‘synthetic genetic array’ (SGA) analysis that automates yeast genetics and enables the systematic construction of double mutants. We developed two powerful pipelines, which combine SGA and automated microscopy for systematic and quantitative cell biological screens or phenomics. Our first pipeline uses SGA to introduce fluorescent markers of key cellular compartments, along with sensitizing mutations, into yeast mutant collections. We then perform live cell imaging on the mutant arrays using HTP confocal microscopy to quantitatively assess the abundance and localization of our fluorescent reporters, providing cell biological readouts of specific pathways and cellular structures in response to thousands of genetic perturbations. Our second pipeline exploits the yeast GFP collection, a unique resource consisting of thousands of strains with different genes uniquely tagged with GFP. This remarkable collection has been arguably underutilized for systematic analysis of the proteome, largely due to the challenges associated with analysis of large sets of cell biological data. We addressed this challenge by adopting a high-content screening approach to measure protein abundance and localization changes in an automated fashion on a genome scale. Our general approach, in particular our network analysis and visualization methods, are readily extensible to other systems.

Convergent targeting of a validatable host-network by pathogens from three kingdoms of life

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Here, we present a systematic physical protein interaction network between virulence effectors of the powdery mildew fungus *Golovinomyces orontii* and *Arabidopsis thaliana* host proteins, which was integrated with corresponding data for *Pseudomonas syringae* and *Hyaloperonospora arabidopsidis*. In the integrated network host proteins can be identified onto which effectors from different pathogens (interspecies convergence) and effectors from each individual pathogen (intraspecies) converge. Systematic phenotypic characterization of genetic deletion lines for *Arabidopsis* target proteins reveals a correlation between intra- and inter-species convergence and the average genetic validation rate. Selected interactions were further validated by demonstration of co-localization, and of functional and physical interaction in planta. The selective pressure imposed by pathogens contrasts with the central host-network position of effector target proteins and their comparative high conservation. Strikingly, when information on natural genetic variation was integrated with the *Arabidopsis* interactome network, the most variable, and positively selected *Arabidopsis* proteins were found as direct interactors of the most targeted host proteins.

From phosphoproteomics networks to new functions in plant receptor kinases

Waltraud Schulze

MPI Potsdam-Golm and Plant Systems Biology, University Hohenheim, Germany

The transmembrane receptor kinase family is the largest protein kinase family in Arabidopsis, and contains the highest fraction of proteins with yet uncharacterized functions. Here, we present functions of SIRK1, a receptor kinase that was previously identified with rapid transient phosphorylation after sucrose resupply to sucrose-starved seedlings. SIRK1 was found to be an active kinase with increasing activity upon external sucrose supply. In *sirk1* T-DNA insertional mutants, sucrose-induced phosphorylation patterns of several membrane proteins were strongly reduced, particularly pore-gating phosphorylation sites in aquaporins were affected. SIRK1-GFP fusions were found to directly interact with aquaporins in affinity pull-down experiments on microsomal membrane vesicles. Furthermore, protoplast swelling assays of *sirk1* mutants and SIRK1-GFP expressing lines confirmed a direct functional interaction of receptor kinase SIRK1 and aquaporins as substrates for phosphorylation. Lack of *SIRK1* expression resulted in a failure of mutant protoplasts to control water channel activity upon change in external sucrose concentration. We propose that SIRK1 is involved in regulation of sucrose-specific osmotic responses by direct interaction with and activation of an aquaporin via phosphorylation and that the duration of this response is controlled by phosphorylation-dependent receptor internalization.

The *TARA*-Oceans project: towards plankton eco-systems biology

Chris Bowler and the Tara Oceans Consortium

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With biology becoming quantitative, systems level studies can now be performed at spatial scales ranging from molecules to ecosystems. Biological data generated consistently across scales can be integrated with physico-chemical contextual data for a truly holistic approach. While the marine planktonic ecosystems that diatoms inhabit comprise the base of the ocean food web, and are crucial in the regulation of Earth's biogeochemical cycles and climate, their organization, evolution and dynamics remain poorly understood. The *Tara* Oceans expedition was launched in September 2009 for a 3-year study of the global ocean ecosystem aboard the schooner *Tara*. A unique sampling programme encompassing optical and genomic methods to describe viruses, bacteria, archaea, protists and metazoans in their physico-chemical environment has been implemented. The project aims to generate systematic, open access datasets usable for probing the morphological and molecular makeup, diversity, evolution, ecology and global impacts of plankton on the Earth system, as well as to explore and exploit their biotechnological potential. Using the unique *Tara* Oceans dataset we are exploring diatom abundance and biodiversity in the world's oceans.

Mapping the transgenerational epigenetic basis of complex traits

Frank Johannes

University of Groningen, Groningen Bioinformatic Centre, Faculty of Mathematics and Natural Sciences, The Netherlands

Quantifying the impact of heritable epigenetic variation on complex traits is an emerging challenge in population biology. Here we analyzed a panel of nearly isogenic *Arabidopsis* lines which segregate experimentally induced DNA methylation changes genome-wide. We provide compelling evidence that a small number of transgenerationally stable differentially methylated regions (DMRs) act as *bone fide* epigenetic quantitative trait loci (QTL^{epi}) in this population, accounting for 60-90% of the observed heritability underlying two complex traits, flowering time and root length. We show that these QTL^{epi} are reproducible and can be subjected to artificial selection. Over 75% of the putative causal DMRs within the QTL interval are also variable in wild populations of this species and are not significantly associated with *cis* or *trans* acting SNPs. These sequence-independent DMRs may be an important source of phenotypic diversity in ecological settings and thus provide a basis for Darwinian evolution.

Engineering phenylpropanoid metabolism for healthier foods

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The past 20 years has seen an enormous rise in publicity about super foods that promote health and reduce the risk of cardiovascular disease, cancer and age-related degenerative diseases, related specifically to the metabolic syndrome. These claims are supported by robust evidence from cell studies, animal feeding trials, human intervention studies and epidemiological studies. However, despite all the positive messages about the value of eating fruit and vegetables (the 5-a-day program has been running for 25 years) the numbers of people meeting these dietary recommendations in the US remains below 25% of the population, numbers are falling, and chronic diseases, especially those associated with obesity and the metabolic syndrome, are reaching epidemic proportions in Western societies.

There is a need to engineer high levels of protective bioactives in the foods that people actually do consume, to help combat this rise in chronic diseases. Most attempts at engineering the levels of bioactives have focused on increasing the activity of key, rate-limiting steps, but such strategies usually result in only modest improvements in flux to bioactive end-products. Use of transcription factors to up-regulate entire pathways of plant secondary metabolism is a far more effective strategy and results in food material with very significantly elevated levels of health-promoting bioactives. While such improvements may, in part, be achievable for some crops through selective breeding, genetic modification offers bigger improvements because it can overcome limits in the natural variation available in transcription factor specificity and activity. Use of genetically improved foods in animal feeding studies with models of tumorigenesis have revealed that protection is afforded by diets enriched in high polyphenol foods. Cellular studies are starting to throw light on the mechanisms of action of dietary polyphenols. Comparison of foods enriched in different polyphenols allows for quantitative assessment of their bioactivity and establishment of synergistic interactions between them.

Quantitative imaging of transport activity and metabolite dynamics with fluorescent biosensors

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Revolutionary new technologies, namely in the areas of DNA sequencing and imaging, continue to impact new discoveries in plant science and beyond. For decades we have been able to determine properties of enzymes and transporters *in vitro* or in heterologous systems, analyze their regulation at the transcriptional level, use GFP reporters to obtain insights into cellular and subcellular localization, and measure ion and metabolite levels with unprecedented precision using mass spectrometry. However, we lack key information on location and dynamics of the substrates of the enzymes and transporters, and on the regulation of the proteins in their cellular environment. Such information can now be obtained by transitioning from *in vitro* to *in vivo* biochemistry using biosensors. Genetically encoded fluorescent protein-based sensors for ion and metabolite dynamics provide highly resolved spatial and temporal information, and are complemented by sensors for pH, redox, voltage, redox, and tension. They serve as powerful tools for identifying missing processes and components and signaling networks, e.g. analysis of glucose transport across ER membranes, identification of SWEET sugar transporters for cellular sugar efflux and systematic screening of mutants that affect sugar transport or cytosolic and vacuolar pH. More recently we have been able to engineer sensors that report the activity of transporters, allowing us to study their activity and regulation *in vivo*. Together, biosensors promise to be key diagnostic tools for systems and synthetic biology.

A peroxidase regulates the flux to anthocyanins and lignin in *Fragaria x ananassa* fruit.

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Plant phenolics have attracted much attention recently due to their assumed nutritional benefits. Although the major enzymes of their biosynthetic pathways in plants have been analyzed in detail, the regulation of their production and flux through the pathway is not that well established. The goal of this study was to use a strawberry (*Fragaria x ananassa*) microarray to reveal gene expression patterns associated with the accumulation of phenylpropanoids, flavonoids, and anthocyanins in strawberry fruit. An examination of the transcriptome, coupled with metabolite profiling data from different commercial varieties, was undertaken to identify genes whose expression pattern correlated with altered phenolics composition. Seventeen pairwise microarray analyses revealed 15 genes that were differentially (more than 200-fold) expressed in phenolics-rich versus phenolics-poor varieties. The results were validated by heterologous expression of the most promising candidate peroxidase FaPRX27 gene, which showed the highest altered expression level (more than 900-fold). The encoded protein was biochemically characterized and is assumed to be involved in lignin formation during strawberry fruit ripening. Quantitative trait locus analysis indicated that the genomic region of FaPRX27 is associated with the fruit color trait. Down-regulation of the CHALCONE SYNTHASE gene and concomitant induction of FaPRX27 expression diverted the flux from anthocyanins to lignin. The results highlight the competition of the different phenolics pathways for their common precursors. The list of the 15 candidates provides new genes that are likely to impact polyphenol accumulation in strawberry fruit and could be used to develop molecular markers to select phenolics-rich germplasm.

Comparative analysis of benzoxazinoid biosynthesis in monocots and dicots

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Plants synthesise a multitude of secondary metabolites. Variability seems to be essential for the function of these “specialised metabolites” in communication with the environment and as an arsenal for chemical defence. Establishment of secondary metabolic pathways is based on the common primary metabolism. Specific enzymes are then involved to generate the species or family specific secondary metabolites. In the genomes large gene families of the modifying enzymes, e.g. cytochrome P450 monooxygenases, 2-oxoglutarate dependent dioxygenases, methyltransferases, glycosyltransferases are present and constitute the “toolbox” from which unique enzymes are recruited. Little is known about the principles to choose a member of this arsenal, to shape it for the requirements of a distinct step in biosynthesis and to coordinate the reaction sequence. Isolation and comparison of biosynthetic genes for pathways that are shared in unrelated plant species can give insight into the mechanism of pathway evolution. Benzoxazinoid biosynthesis is well suited for such an analysis.

Benzoxazinoids are defence-related secondary metabolites found in three orders of the angiosperms: *Poales*, *Ranunculales* and *Lamiales*. These preformed defence compounds control broad spectra of microbial pathogens and herbivores. While the pathway is monophyletic in the grasses the branch point reaction to primary metabolism, the stabilisation of the toxic intermediate and bio-activation evolved independently by recruitment of homologous but not orthologous genes in the dicot and monocots. Our analysis suggests that prior to the separation of the lineages a pool of progenitor enzymes with substrate and reaction ambiguity was existing that allowed independent pathway evolution in the different orders. Enzyme promiscuity might be the basis for generation of secondary metabolite diversity in plants.

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