XVIII. Berliner Botanisches Graduierten-Kolloquium

"Havel-Spree Kolloquium"

Freie Universität Berlin

Biochemie der Pflanzen
Königin-Luise Str. 12-16
14195 Berlin

Freitag 16.11.2007

Freie Universität Berlin

Content

Programm

10:00 Begrüßung/ Welcome speech by Prof. Dr. Tina ROMEIS

Session 1: Stress

10:15 **Katrin STRAßBURG**, MPIMP Golm (AG Kopka)
Metabolome and transcriptome analysis of the temperature stress response of the model yeast, *Saccharomyces cerevisiae*

10:30 **Magdalena MUSIALAK**, MPIMP Golm (AG Scheible)
Investigating novel, potential regulators in phosphate stress responses of *Arabidopsis thaliana*.

10:45 **Sandra FRANZ**, Freie Universität Berlin (AG Romeis)
*At*cpk21 function in salt stress response

11:00 **Ullrich Dubiella**, Freie Universität Berlin (AG Romeis)
Function of calcium-dependent protein kinases (CDPK) during the onset of early plant defence responses

11:15-11.45 Kaffeepause/ Coffeebreak

Session 2: Development and Metabolism

11:45 **Tilbert KOSMEHL**, Freie Universität Berlin (AG Kunze)
Senescence associated members of the ALA gene family in *Arabidopsis thaliana*

12:00 **Christoph EDNER**, Universität Potsdam (AG Steup)
Glucan, water dikinase (GWD) activity enhances breakdown of starch granules by Chloroplastic amylases

12:15 **Susanne BEICK**, Humbold Universität Berlin,
(AG Schmitz-Lineweber)
The pentatricopeptide protein PPR5 is essential for the stability of an unspliced tRNA precursor in maize chloroplasts

12:30 **Undine KRÜGEL**, Humbold Universität Berlin (AG Kühn)
Transport and sorting of the Solanacean sucrose transporters StSUT1 and *Le*SUT1 is affected by redoxdependent regulation

12:45 **Michael FRITZ**, Humbold Universität Berlin, (AG Ehwald)
A cortical pathway of axial water transport in fine branch roots of wetland plants

13:00-14:00 Mittagspause/ Lunch

Session 3: Signalling

14:00 **Helen BRAUN**, Freie Universität Berlin, (AG Schmülling)
Molecular characterisation of repressors of the cytokinin deficiency syndrome

14:15 **Hamad SIDDIQUI**, Universität Potsdam, (AG Müller-Röber)
A novel gene, PHL, regulates senescence in *Arabidopsis thaliana*

14:30 **Henriette WEBER**, Freie Universität Berlin (AG Hellmann)
BPM (BTB/POZ-MATH) protein interactions in *Arabidopsis thaliana*

14:45 **Kerstin HOLST**, Freie Universität Berlin (AG Werner)
Cytokinin deficiency causes distinct changes of sink and source parameters in the shoot

15:00 **Eswar RAMIREDDY**, Freie Universität Berlin, (AG Heyl)
Ectopic expression of the dominant transcriptional silencer gene *ARR1-SRDX* in *Arabidopsis* suppresses pleiotropic cytokinin activities

15:15-15:45 Kaffeepause/ Coffeebreak

Session 4: Bioinformatics

15:45 **Chang YIN**, Humbold Universität (AG Boerner)
Isolation and characterization of a phage-type RNA polymerase gene from *Selaginella moellendorfii*

16:00 **Justus FUESERS**, Technische Universität Berlin (AG Eichler)
Modelling the time- and wavelength resolved fluorescence dynamics in the PBP-Antenna of the phototrophic cyanobacterium *Acaryochloris marina*

16:15 **Nima YAZDANBAKHSH**, MPIMP Golm (AG Fisahn)
How to avoid digging in the dirt

16:30 **Liam CHILDS**, MPIMP Golm, (AG Walther)
SNP calling using *Arabidopsis* genome tiling arrays

16:45 **Xiaoliang SUN**, MPIMP Golm, (AG Walther)
A fundamental relation between biochemical regulation and the covariance structure of highthroughput data

17:00 **Karin OLLIGES**, Technische Universität Berlin (AG Eichler)
Time and wavelength resolved fluorescence spectroscopy of photoinhibited photosynthetic organisms using a novel multi channel photomultiplier system

Im Anschluss/ Afterwards:

Getränke und Snacks in der Lobby/ Drinks and snacks in the lobby

Max-Planck-Institut
für Molekulare Pflanzenphysiologie

Metabolome and transcriptome analysis of the temperature stress response of the model yeast, Saccharomyces cerevisiae

Katrin STRASSBURG
AG Kopka, Max Planck Institute of Molecular Plant Physiology, Golm

Changes of the environment elicit stress specific responses of biological systems. These responses counteract damaging physical effects and help organisms acclimate to changing conditions and are the basis for survival in a species specific range of habitats. In unicellular organisms, such as yeast, these responses utilize complex known metabolic and signal transduction networks, which ultimately may effect the whole cellular machinery. We focus on the temperature specific responses, which are caused by heat and cold stress. To investigate and compare effects of both temperature responses time course analyses at metabolic and transcriptomic system levels were performed. Initially eight time points were chosen which covered potential immediate and long-term responses. Transcript levels were investigated by conventional Affymetrix GeneChip™ technology, whereas gas chromatography coupled to mass spectrometry (GC-MS) was used for metabolic analysis. In contrast to the conventional approach of GC-MS profiling, which involves calculation of response ratios based on a set of reference samples, which are typically analysed in parallel, we developed and optimized the mass isotopomer ratio profiling technology. This technique combines (1) a fully ^{13}carbon in-vivo labelled reference sample, representing yeast cells obtained by feeding pure U-^{13}C-Glucose at 99 atom %, with (2) a non-labelled sample, which was subject to experimental manipulations [1]. The differential mass shift of labelled versus non-labelled metabolites allows differential mass detection of both samples within the same GC-MS analysis, similar to internal standardization using chemically labelled reference compounds. Using in vivo-feeding the direct determination of relative changes of all pool sizes by monitoring of mass isotopomer ratios is enabled. This fact substantially increases the quantitative accuracy of metabolite analyses and subsequent metabolite/transcript co-response analysis.

[1] Birkemeyer *et al*., (2005) Trends Biotechnol. 23:28-33

Max-Planck-Institut
für Molekulare Pflanzenphysiologie

Investigating novel, potential regulators in phosphate stress responses of Arabidopsis thaliana.

Magdalena MUSIALAK,
AG Scheible, Max Planck Institute of Molecular Plant Physiology, Golm

Metabolism depends on inorganic phosphate (P_i) as a reactant, allosteric effector and regulatory moiety in covalent protein modification. To cope with Pi shortage, plants activate a set of adaptive responses to enhance P_i recycling and acquisition. These responses include changes in biochemical processes, secretion of organic acids and synthesis of enzymes that enable acquisition of P_i from insoluble complexes and organophosphates in the soil. Recent microarray studies confirmed known and also revealed novel, phosphate responsive genes involved in metabolism, in the uptake and transport of P_i, ion homeostasis and in other processes related to growth and development (Morcuende *et al*., 2006). The microarrays also revealed a set of genes involved in cellular signalling, including transcription regulators, MAP2K kinases and several genes related to the targeted protein degradation pathway. However still little is known about P_i sensing and signalling in plants.

Based mainly on long open reading frames (ORFs), genome sequencing identified thousands of new genes in Arabidopsis. However, genes coding for small peptides or ORFs for small functional, non-coding RNAs (ncRNAs) have been largely overlooked. Accumulating evidence indicates that such ncRNAs can play critical roles in a range of cellular processes, including chromosomal silencing, transcriptional regulation, developmental control and responses to stress.

In this work we investigate a novel class of RNAs, called orphan RNA transcripts (oRNAs), which originate form the transcriptional active fraction of the genome but so far their function remains unknown (Riano-Pachón *et al*., 2005). We show that expression of some of the transcripts is significantly changed under P_i (and other nutrient) starvation conditions, as well as in Arabidopsis mutants with altered P_i levels (*pho1, pho2*).

In the light of the recent discovery that miRNAs (Bari *et al*., 2006) are also involved in the P_i stress related responses, some oRNAs could be involved in the modification/regulation of the P_i response of Arabidopsis. The study of these oRNAs should contribute to our understanding of the complex network of metabolite signalling in plants.

Freie Universität Berlin

AtCPK21 function in salt stress response

Sandra FRANZ, Joachim KURTH, Anne-Claire CAZALÉ, and Tina ROMEIS

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Calcium-dependent protein kinases (CDPK) are Ser/Thr protein kinases unique to plants and some protists. CDPKs are implicated in the recognition of stress-specific changes in the cytoplasmatic calcium concentrations and their translation into specific phosphorylation patterns for signal transduction (1). They comprise a gene family in plants, which raises the presumption that particular CDPKs participate in different signal transduction pathways. However, only for two CDPK isoforms (NtCDPK2 and MtCDPK1) function in plant defence and symbiosis has been shown, so far (2,3).
CDPKs consist of a highly conserved structure, which includes a N-terminal variable domain, a catalytic protein kinase domain, an autoinhibitory junction domain and a C-terminal calcium- binding domain with four EF-hand motives for calcium-binding. Calcium binding causes a conformational change, which activates the enzyme.
Comparative physiological assays and expression studies between *A. thaliana Col-0* wildtype and a *cpk21* T-DNA insertion line suggest a function of AtCPK21 in the abiotic salt stress response. For further biochemical characterisation of CPK21, the influence of (auto-) phosphorylation and calcium binding on the enzyme activity as well as on the biological function after stress stimulus in planta was assessed. Different versions of AtCPK21 were transiently expressed in *N. benthamiana*. These variants include a kinase inactive version, single EF-hand mutations and double EF-hand mutations in either the C-terminal or N-terminal EF-hands. Our data show that the C- and N-terminal EF-hand pairs differ in their calcium-binding properties and mutations in these EF-hands have different effects on enzyme activities. These data indicate for the first time a different molecular function for C- and N-terminal EF-hand pairs during CDPK regulation.

References:
(1) Harper JF and Harmon AC (2005). Plants, symbiosis and parasites: a calcium signalling connection. Nature Reviews Molecular Cell Biology 6, 555-566
(2) Romeis T, *et al*. (2001). Calcium-dependent protein kinases play an essential role in a plant defence response, EMBO, 20, 5556
(3) Ivashuta *et al*. (2005). RNA Interference Identifies a Calcium-Dependent Protein Kinase Involved in Medicago truncatula Root Development Plant Cell.; 17(11): 2911–2921.

Freie Universität Berlin

Function of calcium-dependent protein kinases (CDPKs) during the onset of early plant defence responses

Ullrich DUBIELLA, Claus-Peter WITTE, and Tina ROMEIS
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Calcium-dependent protein kinases (CDPKs) have been identified as major signalling mediators, which translate (unfavourable) changes in the environment into specific phosphorylation signals and such, initiate appropriate stress response pathways. A direct involvement of CDPK signalling during induction of plant defence responses has only been shown for two tobacco isoforms, *Nt*CDPK2 and *Nt*CDPK3, where biochemical and transcriptional activation was observed. In loss-and gain-of-function experiments, CDPK signalling was correlated with the induction of defence responses and a cross-communication between parallel MAP kinase and CDPK signalling pathways during early states of defence activation was shown.
To corroborate the role of CDPK signal transduction during defence activation by genetic evidence and decipher CDPK function in the context of host and non-host resistance we study identified CDPK isoforms from A. thaliana. The activation of early defence reactions including oxidative burst production, defence gene activation and cell death development is investigated in respective overexpressing and knock-out plants. A potential exploitation of CDPK signalling for a biotechnological approach will be discussed.

Freie Universität Berlin

Senescence associated members of the ALA gene family in Arabidopsis thaliana

Tilbert KOSMEHL
AG Kunze, Free University Berlin, Berlin

Senescence is the genetically programmed last phase of leaf development and responsible for the recycling of nutrients before the cells die. It is a highly regulated process that involves complex morphological and metabolic changes and extensive alterations in gene expression. The mobilization of metabolites and nutrients out of senescing tissues into juvenile, storage or reproductive organs is mostly dependent on the activity of transmembrane transporters. However, to date only very few senescence-associated transporters (SATPs) have been reported. By a genome-wide transcriptional analysis of *Arabidopsis thaliana* developmental leaf senescence many novel, previously unrecognized, SATPs were identified.
Among these are three putative aminophospholipid translocases that show increased transcript levels in rosette leaves during senescence (ALA1, ALA10 and ALA11). They belong to the P4-type ATPases, a new subfamily of the P-type ATPase ion pump superfamily with 12 members. ALA10 and ALA11 are highly similar (86% identity), whereas ALA1 is more distantly related (33% identity).
T-DNA insertion lines for *ALA1, 10* and *11* were identified and, to recognize overlapping or redundant functions, double and triple mutant lines were generated. Mutant lines *ala1* and *ala11* exhibit weakly reduced growth and enter senescence slightly earlier compared to wild type, whereas the *ala10* mutant line shows no obvious phenotype. The *ala10/11* double mutant line starts to senesce earlier than wild type and shows a stunted growth. Remarkably, in the *ala1/10/11* triple mutant line premature senescence is even more pronounced and accompanied by developmental abnormalities. In all mutant lines cold treatment promotes precocious senescence and reduced growth, whereas these symptoms disappear when plants grow at elevated temperatures. These characteristics are consistent with a role of the ALA proteins in membrane reorganization by transport of phospholipids or modification of the lipid bilayer composition during development and stress response.

Glucan, water dikinase (GWD) activity enhances breakdown of starch granules by chloroplastic β-amylases

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Transitory starch is accumulated in chloroplasts throughout the day and is degraded in the subsequent night. The semicrystalline starch granules consist of two glucose polymers: essentially linear amylose and branched amylopectin. The latter contains phosphate monoesterified to the C6 and C3 position of a rather small proportion of the glucosyl residues.
Starch phosphorylation is catalyzed by the enzymes glucan, water dikinase (GWD) and phosphoglucan, water dikinase (PWD). Arabidopsis mutants lacking GWD or PWD are impaired in proper starch breakdown and exhibit significantly increased leaf starch contents with the phenotype of the GWD-deficient plants being more severe.
To explore the link between phosphorylation and breakdown of starch we searched for starch degrading enzymes from Arabidopsis whose activities are affected by glucan phosphorylation. Candidate proteins were enriched from leaf extracts by consecutive chromatographic steps and identified from SDS-gel bands using mass spectrometry.
Among other known starch-related proteins, the debranching enzyme ISOAMYLASE3 (ISA3; At4g09020) and β AMYLASE1 (BAM1; At3g23920) were detected, which are both localized in plastids.
Experiments using purified recombinant enzymes showed that BAM1 activity increased about twofold if the starch granules were simultaneously phosphorylated by recombinant potato GWD. This effect was also observed with BAM3, a chloroplastic BAM isoform that is essential for starch degradation at night. Mixing β-amylases and ISA3 significantly enhanced granule degradation, which was further increased in the presence of GWD and ATP.
β-Amylolytic attack on the granules in turn considerably stimulated the GWD-catalyzed starch phosphorylation. The interdependent activities of GWD and β-amylases offer an explanation for the starch excess phenotype of GWD-deficient plants.

The pentatricopeptide protein PPR5 is essential for the stability of an unspliced tRNA precursor in maize chloroplasts

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One of the largest families of putative RNA binding proteins in angiosperms is the pentatricopeptide repeat (PPR) protein family. This family is greatly expanded in plants (more than 450 PPRs in *Arabidopsis*; only 6 in humans). The vast majority of PPRs are predicted to be targeted to mitochondria or chloroplasts and most of the genetically-characterized PPR proteins influence RNA metabolism in the organelles. However, with few exceptions information on their *in vivo* RNA ligands is lacking. The aim of our group is to combine the determination of *in vivo* RNA targets of some chosen plastid PPR proteins with the analysis of PPR mutants.
Disruption of several PPR genes in *Arabidopsis* leads to embryo lethality demonstrating that those PPR genes are essential for embryogenesis in *Arabidopsis*. Interestingly, a knock out of orthologous genes in maize causes non-photosynthetic plastid ribosome-deficient but viable seedlings in maize. One of the PPR proteins we are working with is PPR5. We could show by RIP-chip assay that the plastid tRNA-G(UCC) is the *in vivo* ligand of PPR5 in maize. Analysis of *ppr5* mutants revealed that the lost of PPR5 protein leads to a severe (nearly complete) reduction of the tRNA-G transcript. We propose that PPR5 is necessary for stability of tRNA-G precursor.
Our results support the idea that plastid PPR proteins essential for embryogenesis in *Arabidopsis* are involved in the synthesis of components of the plastid translational apparatus in maize.

Transport and sorting of the Solanacean sucrose transporters StSUT1 and LeSUT1 is affected by redox-dependent regulation

Udine KRÜGEL
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The plant SUT1/SUC2 sucrose transporters are highly hydrophobic membrane proteins and transport sucrose with high affinity. They have been classified to the group of the glycoside-pentoside-hexuronide (GPH): cation symporters within the major facilitator subfamily (MFS). Oligomerization of transport proteins seems to be a general feature and is assumed to be responsible for the regulation of their transporting properties. Until now, the quaternary structure of plant transporter proteins is not well established. The split-ubiquitin system has been successfully applied to investigate oligomer formation of sucrose transporters from plants (Reinders *et al*., 2002). The quaternary structure of the sucrose transporter proteins StSUT1 from potato and LeSUT1 from tomato plants was elucidated by biochemical methods both *in vitro*, *in planta* and in the heterologous expression hosts *Saccharomyces cerevisiae* and *Lactococcus lactis*. Oligomerization seems to be cell type-specific: in phloem-specific StSUT1 antisense plants, a decrease in the dimeric state of the StSUT1 protein was observed, while the monomeric state remained unaffected. Both, targeting and activity of the sucrose transporter are affected by the redox environment. Localization studies of LeSUT1-GFP showed that an oxidative environment increased the targeting of the protein to `raft-like` microdomains in the plasma membrane. It is known from several other membrane proteins, that targeting to the plasma membrane might be affected by the redox state of the protein.

Ref.: Reinders, A., Schulze W., Kühn C., Barker L., Schulz A., Ward J. M., Frommer W. B. (2002). Protein-Protein Interactions between Sucrose Transporters of Different Affinities Colocalized in the Same Enucleate Sieve Element. The Plant Cell 14: 1567-1577

A cortical pathway of axial water transport in fine branch roots of wetland plants

Michael FRITZ
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The finest branch roots (FBR) of the common reed and other helophytes are restricted to adventitious roots growing at the surface or above the anoxic ground. They do not have an aerenchyma. In contrast, their cortical intercellular spaces form straight channels filled with liquid. An intensive axial volume flow away from the cut of detached FBRs could be observed with the light microscope by displacement of microparticles. When grown on brackish water (140 mM NaCl), osmotically driven exudation from FBRs of reed was observed even at external sodium chloride concentrations up to 220 mM. A Sodium citrate and polyelectrolytes reversed water flow at higher osmotic potential (critical value – 1.5 to -2 bar) than sodium chloride (critical value ~ -10 bar). Exudation on the cortical pathway is more sensitive to osmotic stress than exudation on the stelar pathway. The outer epithelia of the cortex of FBRs of reed are lignified and suberized in a short distance from the tip, whereas the endodermis is lignified at the basis only. The cortical intercellular channels have diameter similar to those of xylem vessels (6 to 8 μm), but occur in much higher number. They increase the conducting cross sectional area by more that 5 times. It may be argued that the decreased resistance of axial flow obtained by the function of this pathway enables efficient hydraulics even at very low root diameter (100 μm and less).

Freie Universität Berlin

MOLECULAR CHARACTERISATION OF REPRESSORS OF THE CYTOKININ DEFICIENCY SYNDROME

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Cytokinins are a class of plant hormones controlling numerous aspects of plant development throughout the life cycle. The catabolic inactivation of cytokinins is catalyzed by cytokinin oxidases/dehydrogenases (CKX) and in many plant species they are responsible for the majority of metabolic cytokinin inactivation.
We carried out suppressor mutagenesis of *35S:AtCKX1*-overexpressing transgenic Arabidopsis plants which display a strong cytokinin-deficient phenotype and screened for mutants that showed a partial reversion of the cytokinin deficiency syndrome. By this approach we aimed to identify genes which are necessary for the establishment of the cytokinin deficiency syndrome, e.g. negative regulatory elements of cytokinin signalling or metabolism.
We isolated several recessive and dominant second site mutations which are being characterised. Using map-based cloning we identified *ROCK2* and *ROCK3* mutant alleles (ROCK = repressor of cytokinin deficiency) as missense mutations in the *AHK2* and *AHK3* genes, which encode for histidine kinase cytokinin receptors. These putative gain-of-function receptor alleles caused an almost complete reversion of the cytokinin-deficient syndromes of *35S:CKX1* plants. Detailed phenotypic analysis of *rock2* and *rock3* single as well as *rock2,3* double mutants in wild-type and *35:CKX1* background revealed partially specific changes in cotyledon expansion, root growth, flower induction and senescence.

A novel gene, *PHL*, regulates senescence in Arabidopsis thaliana

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Leaf senescence is a unique developmental process that involves the transition from a functional photosynthetic organ to an actively degenerating and nutrient-recycling tissue. Senescence in plants is a highly regulated process and depends on the expression of many genes. To identify transcription factors (TFs) induced during natural leaf senescence in *Arabidopsis thaliana* we performed quantitative real-time PCR (qRT-PCR), allowing to analyse the transcript abundance of ca. 1900 TF genes. Several TF genes were found to exhibit enhanced or reduced expression, respectively, in senescent *Arabidopsis* leaves. One of the TF genes selected for further studies was *PHL*. The *PHL* protein was observed to be localised in the cell nucleus when fused to green fluorescence protein (GFP) reporter in a transient expression assay in *Arabidopsis thaliana* protoplasts. Using qRT-PCR, *PHL* was found to be strongly expressed in fully expanded (still green) and 20% senescent leaves, whereas expression was low to moderate in juvenile leaves. Promoter-reporter (GUS) studies confirmed senescence-dependent expression of the *PHL* gene. Constitutive over-expression of *PHL* under the control of the Cauliflower Mosaic Virus 35S promoter caused precocious senescence while RNAi and knock out lines exhibited a significant delay in leaf senescence. *PHL* transcript abundance was not significantly altered in *WRKY53* knock-out lines, indicating that it functions independent of the WRKY53-mediated senescence signalling pathway. Recent experimental data will be presented.

Freie Universität Berlin

BPM (BTB/POZ-MATH) protein interactions in Arabidopsis thaliana

Henriette WEBER
AG Hellman, Freie Universität Berlin

The *Arabidopsis* BTB/POZ superfamily comprises around 80 proteins that can be grouped into 11 subfamilies based on their secondary motifs (reviewed in Stogios et al., 2005; Weber et al., 2007). It was earlier published that the BTB/POZ motif mediates association with other BTB/POZ proteins but also supports interaction with non-BTB proteins such as cullins (Weber et al., 2005). In complex with cullins, BTB/POZ proteins function as substrate adaptors of BRC3 (BTB/POZ-RBX1-CUL3) E3 ubiquitin ligases (Pintard et al. 2003; Wang et al. 2004; Weber et al. 2005). Although basic interaction patterns of BTB/POZ proteins are well explained, hardly anything is known about their biological role in plants.
We are interested in the interaction profile and biological roles of Arabidopsis BPM (BTB/POZ-MATH) proteins that comprise a BTB/POZ subfamily with six members. BPM proteins have a NH2-terminally located MATH domain which is followed by a BTB/POZ fold (Weber et al., 2005). Screening a yeast two-hybrid cDNA library with either full length AtBPM1 or just the MATH domain of AtBPM3, we independently fished 20 different proteins that interacted with both baits.
Two of the found proteins belong to the APETALA 2 (AP2)/ethylene-responsive element binding factors (ERF or EREBP) family of transcriptional regulators that are specifically found in plants (Riechmann et al., 2000). Here I want to introduce a first description on interaction patterns, expression profiles and the overexpression mutant phenotypes of these two BPM interactors, and to point up questions about the possible functional roles of the interaction with BPM proteins.

Cytokinin deficiency causes distinct changes of sink and source parameters in the shoot

Kerstin HOLST[1], Tomáš WERNER[1], Yvonne PÖRS[2], Anne GUIVARC'H[3], Angelika MUSTROPH[2], Dominique CHRIQUI[3], Bernhard GRIMM[1], and Thomas SCHMÜLLING[1]

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Cytokinins are a class of plant hormones, which have a function as key regulators of numerous processes in plant development and growth. Previously we generated transgenic plants overexpressing the cytokinin-degrading cytokinin oxidase/dehydrogenase enzymes from Arabidopsis (AtCKXs). The cytokinin deficiency in these plants caused pleiotropic developmental changes such as the formation of slow growing stunted shoots with small leaves. However, it is unknown which are the cellular processes in growing tissues that limit shoot growth under condition of a reduced cytokinin status. We have investigated whether cytokinin-deficient tobacco plants show changes in different sink and source parameters, which could be causally related to the establishment of the cytokinin deficiency syndrome. Electron microscopy analysis revealed distinct cellular changes indicative of premature differentiation in meristematic cells such as increased vacuolation, decondensation of nuclear chromatin and an earlier differentiation of plastids. Determination of the nuclear DNA content revealed an increased population of cells with a 4C content during early stages of leaf development, indicating a block in the G2 phase of the cell cycle. A comparison of different physiological parameters displayed that despite reduced chlorophyll content, photosynthetic capacity and efficiency as well as the content of soluble sugars were not strongly altered in cytokinin-deficient source leaves, suggesting that cytokinin-deficient plants were not source-limited. In contrast, shoot sink tissues exhibited strongly reduced contents of soluble sugars, decreased activities of vacuolar invertases and a reduced ATP content. These results strongly support a function of cytokinin in regulating shoot sink strength and its reduction may be a cause for the retarded shoot growth upon cytokinin deficiency.

Freie Universität Berlin

Ectopic expression of the dominant transcriptional silencer gene *ARR1-SRDX* in *Arabidopsis* suppresses pleiotropic cytokinin activities

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Cytokinins are essentials to regulate many developmental processes in plants, such as shoot and root development, vascular differentiation, chloroplast biogenesis, leaf senescence and seed development. In *Arabidopsis* cytokinins are perceived and transduced by a variant of the two component signalling system, where the signal is perceived by sensor histidine kinases at the plasma membrane and transported by a phosphorelay via phosphotransmitter proteins to the nucleus where B-type response regulators, a class of transcription factors, are activated. These transcription factors regulate the transcription of their target genes, some of such genes are A-type response regulators, which are involved in the negative feedback in the signalling pathway. We want to investigate the role of B-type response regulators in the cytokinin signalling pathway and their involvement in other plant developmental processes. Eleven B-type response regulators are encoded by the Arabidopsis genome and genetic analyses have indicated functional redundancy. In order to overcome this functional redundancy, we used chimeric repressor silencing technology (CRES-T) and constructed a dominant-negative variant of a B-type response regulator ARR1 by fusing the repressor motif SRDX to the C- terminus of ARR1. The transgenic plants were characterized for consequences on short-term and long-term responses to cytokinin on the morphological and the molecular level. The resultant transgenic shows pleiotropic phenotypes, such as stunted shoot growth, enlarged root system and altered reproductive morphology with early flowering phenotype. The *35S::ARR1-SRDX* plants displayed increased resistance to cytokinin for several bioassays tested, such as primary root elongation, lateral roots formation and detached leaf senescence assays. Molecular analysis indicated attenuation of early transcriptional response to the cytokinin. We also interested to find out the role of B-type ARRs in mediating cross talk with other signalling pathways. The *35S::ARR1-SRDX* plants shows a strong resistance in PhyB-mediated inhibition of germination by far-red light. We further characterized the molecular function of B-type ARRs for the components downstream of this group by performing expression profiling using CATMA arrays. In the end the results clearly demonstrated that B-type ARRs are involved in regulating many, if not most of cytokinin-dependent processes in Arabidopsis and that *35S::ARR1-SRDX* plants are valuable tool for investigating these functions. The results of the microarray data will be discussed.

Isolation and characterization of a phage-type RNA polymerase gene from Selaginella moellendorfii

Chang YIN
AG Boerner, Humbold Universität Berlin

In dicots the phage-type RNA polymerases (RpoT) are encoded by a small family of three genes that is proposed to have evolved by duplication events from the single gene for the mitochondrial RNA polymerase known from all eukaryotes. RpoT polymerases of angiosperms are involved in transcription of mitochondrial and chloroplast genes. We want to know when during plant evolution the gene duplications occurred that led to the complex transcriptional apparatus in higher plants.
Selaginella moellendorfii and some other interesting taxa were selected for this study. Using 3' and 5' RACE techniques, a full-length cDNA sequence of *Selaginella moellendorfii* RpoT was determined. It was also used as a probe for BAC filter Southern hybridization to isolate the genomic DNA *RpoT* sequence. Southern hybridization indicates that there exists only one *RpoT* gene in *Selaginella moellendorfii*. This is surprising as the moss *Physcomitrella patens*, which evolved earlier than *Selaginella*, posses already 3 RpoT genes. Phylogenetic analyses reveal that the *Selaginella* RpoT polymerase is most closely related to the RpoT polymerase from *Physcomitrella*. In phylogenetic trees it does not fall into the branches of well separated plastid and mitochondrial (and dual targeted) polymerase of higher plants, but rather appears within a sister group to the angiosperm RpoTs.
The single *Selaginella* RpoT gene encodes a protein of 1002 amino acids with a predicted molecular mass of 113 kDa. The protein contains a putative transit peptide at its amino terminus. *In silico* targeting predicts a mitochondrial localization. Precise information about the localization is expected from transient expression of RpoT-GFP fusion constructs. Such studies are underway.

Modelling the time- and wavelength resolved fluorescence dynamics in the PBP-antenna of the phototrophic cyanobacterium *Acaryochloris marina*

Justus FUESERS
Laser Group, Prof. Dr.-Ing. Hans Joachim Eichler
Institut für Optik und Atomare Physik
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A. marina discovered only in 1996 has a unique composition of the light harvesting system. The membrane intrinsic chlorophyll (Chl) antenna contains mainly Chl *d* instead of the usually dominant Chl *a* and the membrane extrinsic Phycobiliprotein (PBP) antenna has a simpler rod shaped structure than in typical cyanobacteria [1].
Time- and wavelength resolved fluorescence spectroscopy with a time resolution of 20 ps showed fast excitation energy transfer kinetics of 20-30 ps along the *PBP* antenna of *A.marina* followed by a transfer to the Chl *d* antenna with a time constant of about 70 ps [2].
Calculations of different models describing the energy transfer in the phycobiliprotein (PBP) antenna system of A.marina are compared to the experimental results. With an improved spectrometer system the measurements can easily be performed on living cyanobacterial cells suspended in a cuvette and on whole leafs of higher plants without the need of disrupting the leaf from the plant.

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How to avoid digging in the dirt

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A newly developed platform will be described which allows automatic detection of root growth characteristics for several seedlings simultaneously with high spatial and temporal resolution. A computer controlled positioning unit moves a Petri dish in small increments and enables continuous screening of the surface under a binocular microscope.
Detection of the root tip is achieved by applying thresholds on image pixel data and verifying the neighbourhood for each dark point. Not as the default detection method, image subtraction is applied to detect the growing root tip from any permanent dark object in the background. The growth parameters are visualized as position over time or growth rate over time graphs and averaged over days and light-dark periods by our newly developed software package as well as a 24 hour growth behavior.
Proof of the developed system is provided by application to Arabidopsis starch mutants with severely altered root growth pattern. The results show dramatic decrease of growth rate during dark period in case of sex and *pgm* mutants, while the wild type *col0* seedlings continue growing with almost the same growth rate as during light period.
Application of this system will enable to unravel fundamental questions in plant physiology in an unprecedented manner. In particular, determinants of root growth and thus plant performance can be investigated with highest spatial resolution down to the single cell level. In combination with various mutated plants and the ability to modify the growth media, the system will provide a high throughput detector for improvement of plant nutritional parameters and due to its capacity to simultaneously observe many roots it will furthermore enable a high throughput screening of root growth mutants.

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SNP calling using Arabidopsis genome tiling arrays

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Microarrays enjoy widespread use as a means of investigating the transcriptome of an organism. Recent studies show it is also possible to use them to investigate organisms on a genomic level through the identification of sequence polymorphisms (Borevitz 2006). In the current study, we use whole-genome tiling arrays manufactured by Affymetrix, comprising 2.8 million perfect match and 2.8 million mismatch 25mer probes covering the entire *Arabidopsis thaliana* genome at a regular spacing of 35 nt (center-center). A computational algorithm was developed for calling polymorphic probes based on lowered genomic DNA hybridization signals of a given accession relative to a reference (Col-0) with high confidence. Several variables were tested to ensure the algorithm called SNPs at a maximal rate. The algorithm was validated using known SNPs determined by high-quality sequencing in reference accessions relative to Col-0 (Nordborg et. al. 2005). From tiling chip experiments across 99 ecotypes, we identify an average of 250,000 SNPs equivalent to approximately one SNP every 600 nucleotides per accession. In future research, we plan to associate this genetic information with phenotypic data to identify regulatory genes and regions in the *Arabidopsis* genome.

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A fundamental relation between biochemical regulation and the covariance structure of highthroughput data

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With combined computational simulation and experimental measurements we have recently demonstrated that biochemical regulation is reflected by metabolite correlation network dynamics measured in a metabolomics approach (Steuer et al., 2003; Weckwerth, 2003; Weckwerth et al., 2004; Morgenthal et al., 2006). Here we demonstrate that computergenerated covariance simulation of a metabolic network can be structurally analysed by classical approaches for pattern recognition like principal component analysis. In an iterative approach experimental and computersimulated data are compared to analyse their specific pattern recognition and correlation network topologies.

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Time and wavelength resolved fluorescence spectroscopy of photoinhibited photosynthetic organisms using a novel multi channel photomultiplier system

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The cyanobacterium *Acaryochloris marina* is an unique photosynthetic organism containing mainly Chl *d* instead of Chl *a* in the membrane intrinsic light harvesting systems. These particularities of *A. marina* raise important questions about the mechanism of the excitation energy transfer and the function of Chl *a* and Chl *d* in the reaction centers of *A. marina*. Chl *d* was shown to be the primary donor of the reaction center of PS I in *Acaryochloris marina* (1). However, the pigment composition and the nature of the primary donor (Chl *d* or Chl *a*) in the reaction center of PS II remain unclear. Investigations from Schlodder et al. at TU Berlin (2) and at Kyoto University (3) using respectively different preparations and different spectroscopic methods have yielded contradictory results.
The investigation of photoinhibited cells of *A. marina* showed changes of the Chl *d*-fluorescence at 725 nm due to quenching and slow recombination fluorescence (DF) after photoinhibition similar to what is observed in the Chl *a* fluorescence at 680 nm in typical Cynobacteria or higher plants. The analysis of DF allows to draw conclusions from the primary donor of the reaction center of PS II.
A novel multi channel photomultiplier PML-16C (Becker & Hickl, Berlin) is used for time and wavelength resolved fluorescence measurements of photosynthetic organisms. The photomultiplier detects fluorescence photons simultaneously in the time domain and in 16 wavelength channels. As a result of high count rates up to 10^6 counts/sec very short measurement times are possible.
Therefore this detector can be used for fast monitoring of changes of the fluorescence kinetics appearing after photoinhibition of the photosynthetic organisms with strong white light. Due to the short measurement times it is possible to investigate the dynamics of metabolic changes and repair mechanisms.

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