UPSC Days 29-30 August 2011

Programme

&

Book of Abstracts
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09:50-10:15 Stacey Thompson (Stacey Thompson)
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Book of Abstracts: Presentations
Johannes Hanson

Metabolic Signaling from Utrecht to Umeå

Plant metabolism is dynamic and changed metabolite levels affect growth and development and in an applied perspective yield. We are interested in the regulation of metabolism and the impact of changed metabolite levels on signaling. Specifically we focus on: Sucrose Regulated Translation of bZIP Transcription Factors. Increased sucrose levels inhibit translation of S1-bZIP transcription factors. The mechanism is dependent on upstream open reading frames in the 5’ leader of the messenger. Based on mutation analysis we presented a model in which sucrose dependent stalling of the ribosomes inhibits further translation of the bZIP messenger (Rahmani et al., 2009). Currently we are identifying factors involved in the stalling mechanism by yeast-two-hybrid and immuno-precipitation. Metabolic Reprograming by Sucrose Controlled bZIP Transcription Factors. Over-expression of S1-bZIP transcription factors such as bZIP11 in Arabidopsis dramatically inhibits growth. The main effect of bZIP11 activation is reprogramming of primary metabolism. The levels of several metabolites are affected, including carbohydrates and amino acids (Ma et al., 2011). How the metabolic changes affect growth is under investigation. Fructose Signaling - Revealed by Natural Variation in Arabidopsis. In contrast to glucose, fructose has never been connected in signaling despite its importance in metabolism and high cellular concentration. A locus of the Cap Verde Island accession (Cvi-0) was found to mediate fructose resistant phenotype in a natural variation based screen. Further fine-mapping revealed the Cvi-0 loci to encode a truncated form of the NAC089 gene in which the inhibitory domain is deleted. Overexpressing the Cvi-0 NAC089 gene in sensitive accessions such as Ler and Col-0 mediate fructose insensitive germination phenotype (Li et al., 2011). We are now further characterizing the signaling pathway by determining the in vivo targets of the NAC089 transcription factor.

Currently, we are located in Utrecht (the Netherlands) and moving to UPSC. The talk will serve as an introduction and further details could be found in the posters presented by the group.

References:


Nathaniel Street

Little and Large, Hardwood and Softwood: A Tale of Two Trees
Nathaniel Street

Separated by millions of years of evolution and by orders of magnitude in genome size, aspen and spruce are fascinatingly similar yet different. Both are long-lived, woody tree species yet when bent by snow of the long Umeå winter, one makes tension wood and the other compression wood. To enable us to uncoil such differences, UPSC has established projects to sequence both genomes. Conifers have survived since the time of the dinosaurs yet have seemingly hugely inefficient genomes that are as large as their evolutionary history. At 20 Gbp, Norway spruce has one of the largest genomes on earth. In contrast, aspen represents the spritely youth of the tree world, with one of the smallest tree genomes that harbours amazing levels of variation. As such, both the little and the large of the tree world represent significant challenges to genome assembly.

We are using a next generation sequencing approach to assemble both genomes. To complement genome assembly and annotation efforts, we have additionally undertaken extensive profiling studies of both transcriptomes using RNA Sequencing (RNA-Seq). An overview of both projects will be given with details of the current status of both genome assemblies.
Björn Sundberg (Judith Felten)

Ethylene signaling via Ethylene Response Factors (ERFs) modifies wood development in hybrid aspen

JUDITH FELTENA, JORMA VAHALA, JONATHAN LOVE, ANDRÁS GORZSÁS, LORENZ GERBER, MANOJ KUMAR, JAAKKO KANGASJÄRVI, BJÖRN SUNDBERGA
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The phytohormone ethylene (ET) has the potential to regulate secondary growth of plants. We demonstrated previously that application of exogenous ET or its in planta precursor 1-aminocyclopropane-1-carboxylic acid (ACC) as well as endogenous ET accumulation during leaning stimulate xylem growth in stems of hybrid aspen (Populus tremula × Populus tremuloides) and requires functional ethylene signaling1. Ethylene Response Factors (ERFs) act downstream of ET perception and activate transcription of ET-responsive target genes. We analyze here whether ERFs are regulators of wood development in hybrid aspen.

We identified 169 ERF genes in Populus and studied their responsiveness to ethylene, ACC and tension wood formation in hybrid aspen stems using qPCR. Twenty-six ERFs were expressed in stem tissues and inducible by at least two of the three treatments. Twenty of these ERFs were overexpressed in cambium/xylem in transgenic hybrid aspen but caused only mild alterations of height and radial growth in a greenhouse trial, except for one ERF candidate. A Fourier-Transformed Infra Red spectroscopy and Pyrolysis GC-MS based screening of the ERF-overexpressors revealed changes in xylem cell wall composition (lignin abundance and structure (S:G ratio), glycosidic linkages, cellulose abundance) in xylem tissue. This suggests that ERFs have the ability to modify cell wall composition in wood forming tissues.

Fructokinase is required for carbon partitioning to cellulose in aspen wood

Melissa Roach, Björn Sundberg, and Totte Niittylä

Sucrose is the main transported form of carbon in several plant species including the model tree aspen. Sucrose metabolism in developing wood is therefore central for the regulation of carbon partitioning to stem biomass. Half of the sucrose-derived carbon is in the form of fructose, but metabolism of fructose has received little attention as a factor in carbon partitioning to wood cell walls. We have identified a fructokinase isoform (FRK2), which is important for fructose phosphorylation and carbon flux to cellulose in aspen. RNAi mediated reduction of FRK2 activity in developing wood led to accumulation of soluble neutral sugars and a decrease in hexose phosphates and UDP-glucose indicating that carbon flux to the cell wall polysaccharide precursors was decreased. Reduced FRK2 activity also led to thinner fiber and vessel cell walls with a reduction in the proportion of cellulose, while having no major effect on hemicelluloses. No pleiotropic effects on stem height or diameter growth were observed. The results establish a central role for the FRK2 activity in carbon flux to wood cellulose.
Unlike animals, plants constantly adjust their growth pattern to sustain their needs and adapt to various environmental changes. Plants can achieve this via differential growth, a process involving asymmetric elongation or division of cells within a tissue. The gravitropic response in root and shoot and the phototropic response in the shoot are well studied examples of differential growth. Additionally, differential growth based on intrinsic developmental program occurring in absence of external signals is exemplified by the apical hook formation and maintenance to protect the meristematic primordia from damage during penetration of soil after germination. Regulation of differential growth in plants often involves the differential distribution of the plant hormone indole-acetic acid (auxin). Intracellular polar localization of auxin efflux- and influx- carriers plays a key role in providing directionality to auxin transport to achieve differential distribution of auxin which in turn will act to regulate differential cell elongation. In contrast with gravitropism and phototropism intracellular framework directing the trafficking of auxin carriers involved in differential growth in apical hook formation and maintenance remains poorly characterised. Here, we show that differential trafficking pathways at the post-Golgi compartment called trans-Golgi network (TGN) mediates differential secretion of the auxin influx-carrier AtAUX1 and the auxin efflux-carrier AtPIN3. This process is dependent on the TGN-localized protein ECHIDNA identified previously in the lab. The potential molecular mechanisms involved will be discussed.
Rosario Garcia Gil (Sonali Ranade)

Gene expression analysis in Scots pine in response to red and far-red light

Sonali Ranade, Rosario Garcia Gil

Light is vital for plant growth, morphogenesis and metabolism. Genome expression pattern under red light and far-red light is extensively studied in angiosperms but little is known about the gene regulation in this aspect with reference to Scots Pine. We studied gene regulation in P.sylvestris from northern Sweden grown under red and far-red light using P.taeda cDNA microarrays. Seeds from northern Sweden were grown under continuous red and far-red light under two separate experiments. Microarray was performed with P.taeda cDNA microarrays. Microarray data was normalised by LOWESS normalisation method and by computing the log ratios of the intensity measurements. Statistical analysis was carried out by calculating the t-test statistic and the corresponding p-values from the M-values for each gene in the dye swap experiment. The p-values were adjusted by FDR method using the R-statistical package. The microarray data analysis revealed expression of 405 genes which was enhanced under cR light treatment; while the expression of 239 genes was enhanced under the cFR light treatment. These results indicated that cR light acts as promoting factor whereas cFR antagonises the effect in most of the processes like C/N metabolism, photosynthesis and cell wall metabolism which is in accordance with former findings in Arabidopsis. Differentially expressed genes were annotated using Blast2GO tool.
Karin Ljung

**Root development and shoot-root communication**

The research in my group is focused on mechanisms and processes regulating plant growth and development, and we are particularly interested in the role of different plant growth regulating substances (plant hormones) in primary and secondary root development. We are also interested in understanding how plants coordinate the growth of the aerial parts with the root system, and the roles that plant hormones play in these processes.

Auxin is a key regulatory hormone in plants, controlling basic processes of cell identity, proliferation and organ formation, and acting via strictly controlled relationships between mechanisms setting up physical gradients of the hormone over cell layers, tissues and organs together with signal transduction mechanisms that determine the individual cells responses to the hormone. By using sensitive mass spectrometry-based methods for plant hormone analysis, we have demonstrated the existence of local auxin gradients and maxima/minima in different plant tissues, e.g. in the primary root apex. The formation of the auxin gradient is very important for specifying the root stem cell niche, and for the regulation of cell division and cell differentiation in the root apex. We have showed that the Arabidopsis root apex has a high capacity for auxin biosynthesis and degradation, and we are now trying to understand the molecular mechanisms regulating auxin metabolism.

We are also interested in understanding how auxin interacts with other plant hormones, especially cytokinins. These two hormones sometimes act synergistically and sometimes antagonistically during plant growth and development, and the mechanisms behind these interactions are largely unknown. We have recently shown that auxin and cytokinins can regulate each other’s biosynthesis, and we are currently investigating the mechanisms behind these interactions, and the importance they play for different developmental processes in plants.
Ondřej Novák

**Auxin metabolite profiling**

The homonal profiling methods use modern analytical tools based on fast chromatographic separation and mass spectrometric quantitative analysis. The major problem associated with plant hormone analysis is that the amount of phytohormones present endogenously in plant tissues is very low, usually in the range of fmol to pmol/g fresh weight. On the other hands, the robustness, sensitivity, selectivity, through-put and cost-effectiveness of analysis are also critical factors for convenience of developed method.

The plant hormone auxin is believed to influence almost every aspect of plant growth and development. Auxin transport and metabolism combine to form gradients and maxima/minima in developing cells and tissues, and these dynamic changes in auxin levels are believed to influence specific developmental processes. In order to understand these processes, measurements of steady state auxin levels and biosynthesis rates can give valuable information, and recent advances in instrumentation and method development makes it now possible to quantify auxin at cellular resolution. There is abundant genetic evidence for the existence of multiple pathways for auxin biosynthesis and degradation. This redundancy makes it difficult to understand the relative importance of specific metabolic pathways during development.

We have now developed a method for profiling the majority of known auxin precursors and conjugates/catabolites in small amounts of *Arabidopsis* tissue. Our method includes trace analysis of 21 compounds with different polarity, acidity and basicity as well as stability and abundance in crude plant extract. We found that a using of polymer based (Oasis HLB) sorbents was the best tool in the one-step purification, giving the average total extraction yields close to 85%, 60% and 50% for 0, 10, and 20 mg of fresh plant weight, respectively. The process was completed by a single chromatographic analysis of auxin metabolites in 12 minutes using an analytical column packed with sub-2-microne particles. In multiple reaction monitoring mode, the detection limit for most of analytes ranged from 1.0 to 5.0 fmol and achieved linear range was at least five orders of magnitude. Finally, the accuracy and precision of the method was validated using spiked samples of 10-day-old *Arabidopsis thaliana* seedlings.

We have then used this method to profile different tissues from *Arabidopsis* ecotypes and mutant lines, and can show that there are substantial differences in metabolite levels. We hope that by using this method, we will have a tool to get a better understanding of the regulation of auxin metabolism during plant development.
Annita Sellstedt (Rasika Kudahettige)

Bioethanol Production from Hexoses and Xylose by the White Rot Fungus *Trametes versicolor*

Rasika Kudahettige, Anita Sellstedt

Bio-ethanol production by white rot fungus (*Trametes versicolor*), identified from fungal mixture in naturally decomposing wood samples, from hexoses and xylose was characterized. Results showed that *T. versicolor* can grow in culture, under hypoxic conditions, with various mixtures of hexoses and xylose and only xylose. Xylose was efficiently fermented to ethanol in media containing mixtures of hexoses and xylose, such as MBMC and G11XY11 media, yielding ethanol concentrations of 20.0 and 9.02 g/l, respectively, after 354 h of hypoxic culture. Very strong correlations were found between ethanolic fermentation (alcohol dehydrogenase activity and ethanol production), sugar consumption and xylose catabolism (xylose reductase, xylitol dehydrogenase and xylulokinase activities) after 354 h in culture in MBMC medium. In a medium (G11XY11) containing a 1:1 glucose/xylose ratio, fermentation efficiency of total sugars into ethanol was 80% after 354 h. Presentation will also include ongoing research activities and future perspectives.

**Keywords** Bioethanol, Hexoses and xylose, Hypoxic condition, *Trametes versicolor*, Xylose catabolism
Receptor–like kinases (RLKs) act as cell surface receptors perceiving and transmitting information in various organisms. Plant RLKs regulate growth, development, and biotic interactions, but the specific functions of the majority of plant RLKs remain elusive. We discovered that mutations in the RLK STRUDELEIG-RECEPTOR FAMILY 6 (SRF6) led to changes in DNA methylation in cell-wall-defective Arabidopsis plants and restored the cell wall defects. These epigenetic changes were accompanied by increased expression of COBRA encoding an essential component of cellulose biosynthesis and cell wall establishment in plants. Once an epigenetic state was created, it was transmitted to the progeny independent of SRF6 function. Mutation in the DNA demethylase ROS1 altered COBRA expression similarly to srf6 suggesting a DNA methylation-dependent mechanism for establishment of the epigenetic state. Indeed, whole-genome bisulphite sequencing identified 65 loci with increased methylation and 124 loci with decreased methylation established upon srf6 mutation in response to cobra defect. Our results demonstrate a new role for RLKs in the maintenance and inheritance of epigenetic traits and reveal a previously unknown trigger for epigenetic modification.
Gunnar Wingsle (Robert Nilsson)

Metacaspase Substrate Screening using Filter Aided Sample Preparation

Nilsson, R.¹, Bollhöner, B.², Wingsle, G.¹, Tuominen, H.².

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² Department of Plant Physiology, Umeå University, Umeå Plant Science Centre, SE-901 83 Umeå, Sweden

Background Metacaspases are proteases essential for programmed cell death in plants, though most of their substrates remain unknown (1). The activity of metacaspases is a key issue in understanding wood (dead xylem cells) formation in trees. Here, we performed a substrate screening using a recombinant metacaspase that have an expression profile associated with the programmed cell death during wood formation in poplar.

Methods Substrate preparation was initiated by total protein extraction from xylem tissue of plants down-regulated (RNA interference) in the expression of the metacaspase gene. Proper conditions intended for screening of the enzyme substrates were achieved by combinations of enzyme and substrate ultrafiltration. Utilizing the low pH activation of the enzyme allowed prompt activation and minute monitoring of newly formed peptides. Peptides were analysed by LC-MSMS using a nano-LC system coupled to a Synapt G2 mass spectrometer. Additional extractions including wild-type plants were performed to support detected substrate processing.

Results The results revealed an enzyme active at low temperature (< 7 °C) with properties fitting criteria for cold-adapted enzymes. High specific activity was detected at low temperature and degradation products from the enzyme were formed after 30 min incubation at room temperature.

Conclusions Our preliminary data suggest a substrate that has global responses that would clarify the metacaspase involvement in programmed cell death and wood formation.

Harry Wu (David Hall)

Forest tree genetics and future breeding: from inbreeding to genomic selection

Forest tree breeding, particularly in species in the boreal zone, is time consuming and has been effective for a limited time compared to agricultural crops. There are several issues of breeding effectiveness and speed that current research and technology can improve. Currently the breeding population and climate zone for deployment are not optimized. By using bio-geo-climatic data we can redefine the breeding zones to increase production. Simulation software such as METAGENE can be used to establish new breeding strategies for higher genetic gain, for example, decouple negatively correlated traits and study possible purging of inbreeding depression. Furthermore, establish the economic weights for solid wood, pulp and bioenergy and how to select for those traits are also important in developing breeding objectives more in tune to today's industry. The rapid progress in high throughput sequencing and SNP scoring can be utilized by applying dense marker maps to capture most genetic variation in traits of interest. These markers can then be utilized in an extended form of marker assisted selection termed Genomic Selection. Genomic selection has great potential in speeding up the breeding process and increase genetic gain in traits that are difficult and/or expensive to measure.
Stephanie Robert (Ash Haeger)

Abstract UPSC Days 2011  Ash Haeger

The chemical genomics approach uses small molecules to modify or disrupt the function of specific proteins. It provides a novel avenue for rapid and effective dissection of biological mechanisms and gene networks in ways not feasible with mutation-based approaches. Vesicular trafficking is an essential cellular process driving the distribution of cargos within cells and maintaining subcellular structure. It basically underlies all cellular functions and can be modulated by both developmental and environmental signals. Despite the tremendous importance of this process, our knowledge of the underlying mechanisms and the regulatory networks is very limited. In this study, we have developed a pollen-based high-throughput screen to select chemicals as inhibitors of pollen germination, a process absolutely dependent on intact vesicular trafficking. Among these bioactive compounds, we isolated one chemical, 5-bromo-N-(4-nitrophenyl)-2-thiophenesulfonamide (also referred to as F07). Preliminary data suggests F07 may be an inhibitor of endocytosis. In addition, F07 is acting as a jasmonate antagonist and thus presents some potential to provide novel insights into the connection between the endomembrane system and the regulation of the jasmonate signaling pathway.
Edouard Pesquet (Henrik Serk)

Identification of gene and protein candidates controlling lignin synthesis and polymerization

Henrik Serk, Irene Granlund, Walter Chitarra, Delphine Menard and Edouard Pesquet

Lignin is a phenolic polymer deposited in the cell wall of specialized cells such as xylem tracheary elements (TEs) which form the water and mineral conducting vessels of land plants. Lignin has multiple functions as it provides additional mechanical support to plant organs, cell wall impermeability and a structural barrier against pathogens [1]. Recently, live cell imaging of TE formation showed that lignification occurred after TE programmed cell death (PCD) suggesting that lignin results from a distinct and novel mechanism [2]. In order to address how lignification is controlled at the cellular and sub-cellular level, we are using the Arabidopsis in vitro TE differentiation system in which lignified TEs can be induced by adding hormones [2]. Using confocal laser scanning microscopy and biochemical quantification, we confirmed that lignification occurred after PCD by pharmacologically inhibiting TE PCD or lignin monomer synthesis. Biochemical quantification of phenolic compounds revealed an increase of both intracellular and extracellular phenolics, mostly glucosides, in TEs pharmacologically inhibited to lignify. HPLC analysis showed that specific phenolics were accumulated in both intracellular and extracellular space of TEs inhibited or not to lignifying. Using semi-quantitative RT-PCR, we monitored the expression of all genes of the common phenylpropanoid pathway which produces the lignin monomer precursors. Interestingly, none of the gene tested showed any strict specificity to TE differentiation nor a correlation with produced phenolic compounds. As only 30-40% of cells transdifferentiate into TEs, a novel cell sorting method combining cell protoplastisation and sucrose gradient centrifugation was optimized to separate TEs from non-TE cells. Interestingly, gene expression analysis using RT-PCR showed that lignin monomer synthesis genes were expressed in both TEs and non-TE cells. To localize these genes/proteins at the sub-cellular level, all of the full genomic fragments have been cloned into entry vectors using the GATEWAY system to produce stable fluorescent protein tagged transgenic lines. Preliminary observation showed that lignin monomer synthesis genes were localized in the cytoplasm, around the nucleus and in vesicles. Together our results confirm that lignin synthesis occurs in a partially cooperative fashion where both TEs and non-TE cells produce and export lignin monomer glucosides to the apoplast which will then be used by the TE cell wall proteins to form lignin. Furthermore, our results suggest that the production of lignin monomers is dependent on either 1) a post-transcriptional regulation of the biosynthetic genes/proteins and/or 2) specific association/compartmentalization of these proteins during lignifying conditions.


Book of abstracts: Poster Sessions
Jeroen Lastdrager

Identification of proteins interacting with the Sucrose Control peptide of bZIP11 to understand sucrose signaling

Jeroen Lastdrager¹, Maureen Hummel¹, Jolanda Schuurmans¹, Sjef Smeekens¹,² and Johannes Hanson¹,²,³

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Changed cellular sugar levels are dramatically affecting gene expression in plants. The bZIP11 transcription factor plays a part in this regulatory pathway by affecting genes encoding key enzymes in primary metabolism, thereby acting as a dominant regulator of metabolism (1). In response to high sucrose levels, bZIP11 is translationally repressed, which depends on the sucrose control (SC) peptide encoded by an upstream open reading frame (uORF) in the 5′-leader of bZIP11 mRNA (2). A likely model includes stalling of ribosomes on the bZIP11 mRNA due to sucrose-dependent interactions of the translated SC-peptide with ribosomal or ribosome associated factors (3). This regulatory principle is well conserved and unique to plants.

Transgenic Arabidopsis lines expressing an immuno-tagged SC peptide are being developed, allowing the enrichment and identification of interacting proteins. Additionally, a Yeast-2-Hybrid screening approach yielded several possible protein interactors of the SC-peptide. These experiments could lead to the identification of proteins or protein modifications involved in sucrose dependent stalling of translation and sugar signaling mechanisms in plants.

Jingkun Ma

The Arabidopsis transcription factor bZIP11 reprograms sugar metabolism

Jingkun Ma, Micha Hanssen, Krister Lundgren, Lázaro Hernández, Thierry Delatte, Andrea Ehlert, Chun-Ming Liu, Henriette Schluempmann, Wolfgang Dröge-Laser, Thomas Moritz, Sjef Smeekens and Johannes Hanson

The Arabidopsis transcription factor bZIP11 severely inhibits growth when over expressed. bZIP11 is likely involved in sugar status sensing processes, as sucrose specifically represses its translation. Besides, bZIP11 is known to act downstream of SnRK1 kinase, regulating amino acid metabolism. Furthermore, it is suggested that bZIP11 has a broader regulatory effect in metabolism. Here, we employed large-scale metabolomic and transcriptomic approaches to analyze the regulatory effects of bZIP11 by using bZIP11 dexamethasone nuclear translocation inducible lines. Upon induction, bZIP11 reprograms sugar metabolism rapidly. Moreover, we identified that bZIP11 regulates trehalose metabolism likely via transcriptional activation on several corresponding metabolic genes, TRE1, TPP5 and TPP6. Over-expression of bZIP11 rescues the growth inhibition caused by exogenously applied trehalose, partly due to the induced expression of trehalose (TRE1). Importantly, bZIP11 induction lowered the contents of trehalose 6-phosphate, which has been proposed as signaling molecule. These findings indicate a possible interaction between two sugar status sensing systems which involve trehalose 6-phosphate and SnRK1 respectively.
Melis Kucukoglu

A WUSCHEL-like Gene Controls Stem Secondary Growth in Trees

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Wood formation starts with cell division and differentiation in a secondary meristem called vascular cambium, which forms a continuous cylinder of meristematic cells in the stem. Although many anatomical studies have been performed on the cambial zone and its derivatives, very little is currently known about the molecular and genetic mechanisms regulating the maintenance and differentiation of these stem cells as well as the patterning during the secondary growth of the woody plants. Here we investigate the role of a WUSCHEL-like gene PtHB3 during secondary growth in Poplar. In the transgenic plants expressing an RNAi construct targeting the PtHB3 gene, the width of the vascular cambium was severely reduced and the secondary growth was severely diminished, showing that PtHB3 controls the cell identity and division activity in the vascular cambium. Moreover, ectopic expression of a Poplar CLE41/44-like (CLAVATA3/ESR-RELATED 41/44) gene in trees caused defects in the establishment of cambial cell divisions and the patterning of the vascular tissues. Based on the transcriptional data, a positive feed-forward loop involving PtHB3, PtCLE41 and the receptor-like kinase gene PtRLK3 is suggested to regulate the identity and activity of the vascular cambium.

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Maria Barciszewska-Pacak

Elucidation of the Function of an Alternatively Spliced Gene in Vascular Development in Arabidopsis

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BACKGROUND

Elucidation of a function of important genes is a priority of a post-genomic research. Close to 50% of genes highly expressed in wood forming tissues are unknown [1]. Poplar transcriptomics analyses across the wood developmental zones let us identify with high precision the genes expressed at specific stages during wood cell development [2]. A landmark of wood cell development that distinguishes it from other cells in plants is the onset of the secondary wall formation. Recent studies identified a set of transcriptional regulators involved [3], but beyond that, our knowledge of the transition to secondary wall formation is very poor.

RESULTS

In poplar, many unknown function genes are specifically expressed at the onset of secondary walls. We have identified Arabidopsis orthologous genes and selected 17 genes in Arabidopsis and for functional analyses (Fig. 1).

The phenotypical characterization of Arabidopsis lines carrying promoter-GUS fusions, T-DNA tag insertions in the coding regions, 35S:cDNA constructs and RNAi suppression constructs resulted in the identification of an alternatively spliced gene A with unknown function that was causing striking “bushy” phenotypes when altered. The implementation of a new method of induced xylogenesis in transformable cell suspensions [4] revealed its role in xylogenesis. The predicted gene A structure consists of 12 exons. We have carried out extensive 5’ and 3’ RACE experiments from exons 1, 4 and 11 to identify all splice variants of gene A. Two main forms of transcripts were detected, although several splice variants e.g. short transcripts with exon 1 and entire or truncated exons 2 and 3 are implied. The long form contained all 12 exons with at least 4 potential 3’UTRs. There was detected also potential long transcript form with several introns retained. Two 5’UTR forms detected were equally common. The existence and expression of gene A splicing variants and their importance for the observed plant phenotypes are being studied.

CONCLUSIONS

Gene A is an alternatively spliced gene of unknown function that has a complicated pattern of transcript processing. The observation of the changed expression phenotypes of the gene A brings support for its importance for plant development and xylogenesis.

REFERENCES

Julia Wind

Fructose sensitivity is suppressed in Arabidopsis by the transcription factor ANAC089 lacking the membrane bound domain

Sugar repression of seedling development was used to study fructose sensitivity in the Landsberg erecta (Ler)/Cape Verde Islands (Cvi) recombinant inbred line population, and FSQ6 was confirmed to be a fructose-specific QTL by analyzing near-isogenic lines in which Cvi genomic fragments were introgressed in the Ler background. These results indicate the existence of a fructose-specific signaling pathway in Arabidopsis. Remarkably, fructose specific FSQ6 downstream signaling interacts with abscisic acid (ABA)- and ethylene-signaling pathways, similar to HXK- dependent glucose signaling. The Cvi allele of FSQ6 acts as a suppressor of fructose signaling. The FSQ6 gene was identified using map based cloning approach, and FSQ6 was shown to encode the transcription factor gene Arabidopsis NAC domain containing protein 89 (ANAC089). Controlled proteolytic activation of membrane-bound transcription factors (MTFs) is a versatile way of rapid transcriptional responses to environmental changes in plants. Amongst NAC (NAM/ATAF1/2/CUC2) transcription factors, at least 45 are considered MTFs, of which ANAC089 is one. The Cvi allele of FSQ6/ANAC089 is a gain-of-function allele caused by a premature stop in the third exon of the gene. The truncated Cvi FSQ6/ ANAC089 protein lacks a membrane association domain that is present in ANAC089 proteins from other Arabidopsis accessions.
Prashant Pavar

Heterologous expression of fungal acetyl xylan esterase (CE1) in Arabidopsis:

Xylan is the third most abundant biopolymer found on the Earth and it contributes to large amount of biomass available for human exploitation. Xylan backbone consists of β-(1 4) linked D-xylopyranosyl residues substituted with 4-O-methyl-D-glucuronic acid/glucuronic acid. The xylopyranosyl residues are partially acetylated in the C-2 and/or C-3 positions. Xylan acetylation might affect the conversion of lignocellulosic biomass to fermentable sugar, which is a crucial step in biofuel production, and it is important for xylan physico-chemical properties. Our aim is to understand intricate mechanism of xylan interactions in cell wall and to develop plants with improved characteristics for the production of biofuel and cost effective processing of biomass.

In our project, we have overexpressed fungal acetyl xylan esterase (CE1) in Arabidopsis to modify xylan acetylation. It was possible to overexpressed CE1 in Arabidopsis with no major visible morphological effect. We found reduced acetylation in mutant lines as compared to WT by FTIR and MALDI. Pyrolysis data showed that mutant lines are significantly different from WT. Saccharification was also improved in mutant lines as compared to WT.
Lorens Gerber

A High-Throughput Screening Platform for Transgenic Plant Research: Pyrolysis-Gas Chromatography / Mass Spectrometry (Py-GC/MS) with Multivariate Curve Resolution Data Processing

Lorenz Gerber(a), Mattias Eliasson(b), Johann Trygg(b), Thomas Moritz(a), Björn Sundberg(a)
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Today, most functional genomic workflows include at some state a high-throughput chemical fingerprinting or chemotyping method and plant science makes no exception. Traditionally Fourier Transform Infrared Spectroscopy (FTIR) has been a popular method for such application. We present here an alternative approach based on pyrolytical sample decomposition followed by GC/MS analysis. Some of its main benefits simple sample preparation and low sample consumption together with the availability of auto samplers make this technique and ideal approach for large scale screening projects. Compared to FTIR, this technique yields additionally to a fingerprint also lignin composition ratios (S/G/H). To cope with the vast amount of data collected by Py-GC/MS analysis, automated multivariate curve resolution was integrated in the workflow. Classification of the processed data is obtained by OPLS-DA.
Yogesh Mishra

Metabolic profiling reveals a metabolic shift in Arabidopsis plants

grown under different light conditions Yogesh Mishra1,2, Hanna Johansson Jänkänpää1, Christiane Funk2, Wolfgang Peter Schröder1,2 and Stefan Jansson1* 1. Umeå Plant Science Centre, Department of Plant Physiology, Umeå University, SE- 901 87 Umeå, Sweden 2. Umeå Plant Science Centre, Department of Chemistry, Umeå University, Umeå, Sweden*Author for correspondence: Stefan Jansson, Tel: +46-90 786 53 54; Fax: +46- 90 786 66 76; Email: stefan.jansson@plantphys.umu.se ABSTRACT: Plants have a tremendous capacity to adjust their morphology, physiology and metabolism depending on variation in growing conditions, this is called phenotypic plasticity. Therefore, it is utmost important to understand study their metabolism and adaptation capacity also natural condition (where they actually developed), not just in growth chambers. In previous study (Mishra et al, submitted) we have studied and quantified how that Arabidopsis grown in field differ from those grown in growth chamber in terms of, for example, leaf morphology, photosystem components and photosynthetic regulatory processes. The metabolome provides another tool for understanding the function and plasticity of plants. Therefore, to achieve insights in above differences into the metabolic level, we compare the metabolite profiles of leaves of wild type Arabidopsis thaliana (Col) growing under constant laboratory conditions and field. Using GC-MS. The data are confounded by the within- and between-day variation in weather the field, but still many metabolites accumulate to very different levels. In particular many sugar and sugar derivatives (fructose, sucrose, glucose, galactose and rafinose) showed very large differences. Also, the levels of aminoacides were in general much higher in field grown plants. Several intermediates of TCA cycle including succinate, fumarate and malate three fold higher in growth chamber compared to field grown plants. Apparently, large metabolic shifts are induced in different growing conditions and our study provides new insight into the mechanism of plant adaptation at metabolomic level, and highlights the role of known protectants under natural conditions.
Pernilla Lindén

Methods and Applications of Fluxomics

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Abstract:

Successful engineering of plant metabolism is an important key to meet the increasing demands for food and energy in the future. A tool for measuring the changes of the metabolic network of a plant would be a great step forward.

The aim of this project is to study metabolic fluxes in primary carbon metabolism of plants with emphasis on subcellular compartmentation. The idea is to combine a labelling method of the photosynthetic metabolites, with mass spectrometry applications such as GC-MS and LC-MS which will provide information that can be used to calculate and design a model of the flow of metabolites in the plant.

The model system of choice is Arabidopsis thaliana (Columbia 0) due to its favourable qualities such as being thoroughly studied and well described in the literature but also due to its fast reproduction, neat size and humble cultivation demands.

Labelling will be done with $^{13}$CO₂ under photosynthetic steady-state conditions.

Method development and application will focus on metabolic flow at the key branching points of the primary carbon assimilation pathways.
Benjamin Bollhöner

Functional Characterisation of Metacaspase9

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Xylem cell death is a crucial developmental process during xylem tissue differentiation in plants. While the key regulators of animal apoptosis, the caspases, were not found in plants, there are structurally related proteins named metacaspases in plants that were suggested to be ancestors of caspases and being involved in corresponding processes in plants.

We have identified two metacaspase genes as candidates for such key regulators in Populus trees on the basis of specific upregulation during the xylem cell death phase of wood formation. The closest Arabidopsis homologue to these two Populus genes is METACASPASE9 (AtMC9, At5g04200). Also AtMC9 shows xylem specific expression pattern throughout the plant, suggesting involvement in xylem cell death. In order to characterise the function of AtMC9 in xylem development, a reverse genetic approach was taken. AtMC9 T-DNA knock-out lines showed rather normal progression of protoxylem differentiation and PCD in in vitro grown seedling roots by analysis of genetic crosses to various xylem reporter lines. However, detailed analysis by electron microscopy revealed a slight delay in post-mortem clearance of the protoxylem vessel elements of the primary root, supporting a role for AtMC9 in the autolytic processes during xylem PCD. Interestingly, more severe alterations in overall growth of the plants and in xylem differentiation were observed in RNAi lines where in addition to a strong suppression of AtMC9 expression also other MCII type metacaspases were slightly suppressed. We are proceeding with a double mutant approach to identify metacaspase genes that may act together with AtMC9 in the regulation of xylem differentiation.

To gain more understanding of the processes AtMC9 is involved in, we are performing a tissue specific proteomic approach, which will help to identify xylem specific METACASPASE9 substrates. Furthermore, a search for upstream regulators of AtMC9 expression is ongoing using an EMS mutagenised GFP reporter gene line. Mutants with alterations in GFP expression patterns, ectopic as well as reduced expression have been identified and will help to understand regulation of AtMC9.
Moritaka Nakamura

SGR9, a RING type E3 ligase, modulates the interaction between amyloplast and F-actin in gravity sensing.

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Gravitropism is initiated by sensing the directional change of gravity within specialized gravity sensing cells. In Arabidopsis shoot, the endodermal cell that contains amyloplasts is essential for gravity sensing. The amyloplast sedimentation toward the direction of gravity is important process to trigger subsequent processes for sensing.

The Arabidopsis sgr9 (shoot gravitropism 9) mutant exhibited weak shoot gravitropism. The SGR9 gene encoding a RING finger protein was expressed in several tissues including shoot endodermis. Complementation analysis by using endodermis specific promoter SCR revealed that SGR9 has function within endodermis. Interestingly, SGR9 is localized to amyloplast within endodermal cell, and exhibited ubiquitin E3 ligase activity in vitro. These results suggest that SGR9 function as ubiquitin E3 ligase on amyloplast within endodermal cell in gravitropism.

Our previous report has shown that some amyloplasts in wild type dynamically move in F-actin dependent manner and some amyloplasts sediment toward the direction of gravity. Amyloplast sedimentation occurs in F-actin independent manner. In sgr9, most amyloplasts dynamically move around the cell and did not sediment to the bottom of the cell. Interestingly, this cytological phenotype as well as gravitropic phenotype of sgr9 was restored by the disruption of F-actin. Live cell imaging of amyloplasts and F-actin showed that the interaction between amyloplasts and F-actin is aberrant in sgr9. Clusters of amyloplasts entangled in F-actin was observed only in sgr9. These results suggest that SGR9 modulate the interaction between amyloplasts and F-actin, probably by promoting release of amyloplast from F-actin. Amyloplasts released from F-actin by SGR9 function may have ability to sediment toward the direction of gravity, leading to subsequent sensing processes. Degradation of unknown substrates by SGR9 E3 ligase activity might be required for the release of amyloplasts from F-actin.
Bastian Brouwer

The light-dependent induction of leaf senescence

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Abstract

Often, leaves have to deal with reduced light-conditions, generally resulting from shading by neighboring plants. As a common response, they can relocate nutrients from shaded to developing leaves through a process called leaf senescence. Shade- and dark-induced leaf senescence are thought to be initiated by either the relative increase of far-red light on phytochromes or the light dropping below the light compensation point. However, the regulatory mechanisms by which these factors induce leaf senescence are still unclear.

We investigated this matter by shading individual attached leaves and analyzing the resulting processes by physiological measurements, transmission electron microscopy and the expression of senescence-associated genes. The results showed that mild shading led to photosynthetic acclimation within the shaded leaf, whereas strong shading led to leaf senescence when the light intensity was lower than the acclimated light compensation point. Furthermore, using phytochrome null-mutants, we showed that despite an increase in chlorophyll-degradation upon shading, these mutants did not increase the expression of senescence-associated genes. Altogether, our results suggest that the induction of shade-induced leaf senescence mainly depends on the efficiency of carbon fixation rather than on a phytochrome-dependent signaling pathway.
Claudia Leoni

Lhcb2 mediates the first phase of State Transition

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The light harvesting complex II (LHCII), associated with photosystems II (PSII), contains three highly homologous chlorophyll-a/b-binding proteins (Lhcb1, Lhcb2 and Lhcb3), which can be assembled in both homo- and heterotrimers. Those proteins are highly conserved during the evolution suggesting that each of them has a specific function. The most abundant of the LHC proteins are the Lhcb1 and Lhcb2 proteins that can be reversibly phosphorylated by the Stn7 kinase to regulate PSI/PSII excitation energy balance in the so-called State Transition process.

To investigate the role of phosphorylation of Lhcb1 and Lhcb2 proteins, we have developed specific antibodies against phosphorylated and non-phosphorylated Lhcb1 and Lhcb2. Phospho-Lhcb1 and phospho-Lhcb2 as virtually absent in “State 1 conditions”, but Lhcb2 is more rapidly phosphorylated than Lhcb1, only 10 seconds of “State 2 conditions” results in phosphorylation of 20 % of Lhcb2, and the process has almost gone to completion after 1 minute of illumination. Lhcb1 phosphorylations, in contrast, take several minutes to complete. The dephosphorylation kinetics of the two proteins is similar. We also used Blue-native electrophoresis to determine the localization of phospho-Lhcb1 and phospho-Lhcb2 among different supermolecular complexes. Most phospho-Lhcb1 and phospho-Lhcb2 was migrating in the trimeric LHCII fraction, but no phospho-LHC was found to associate with PSII supercomplexes. A large fraction of phospho-Lhcb2 did associate with PSI complexes, but in the Psal mutant, who exhibits hyperphosphorylation of LHCII, also phospho-Lhcb1 associated with PSI. This shows that Lhcb2 is mediating the first phase of state transitions, while Lhcb1 is acting on a slower time scale.
Stefano Pietra

Messing with Polarity. The *kreuz und quer* mutant is defective in root hair polarity and trichome orientation

Stefano Pietra, Lothar Kalmbach, Yoshihisa Ikeda and Markus Grebe

Coordination of cell polarity in a tissue layer (planar polarity) is a critical developmental process in diverse multicellular organisms. Polar emergence of root hairs and proximo-distal orientation of leaf trichomes in the model organism *Arabidopsis thaliana* are ideal systems to study planar polarity in plants. We isolated the *kreuz und quer* (*kuq*) mutant in a genetic screen because of its clearly misplaced root hairs. We fine mapped the mutation to a single deletion in the *KUQ* gene and characterized several T-DNA insertion lines, which displayed the *kuq* phenotype and didn’t complement the *kuq* mutation. Interestingly, the large predicted KUQ protein contains two regions that appear conserved in proteins from diverse eukaryotes, whose function is however unknown. The defects of *kuq* could be associated to the distribution of the hormone auxin, critical in planar polarity establishment in plants. In support to this hypothesis, we observed that the asymmetric plasma membrane localization of the auxin transporter PIN2 was altered in *kuq* mutants. Unraveling the function of KUQ and studying its interaction with other players of the planar polarity genetic framework will allow us to integrate this new component in the network and to shed light on new potential domains conserved in eukaryotes.
Annika Johansson

Analysis of isotope labeled amino acids by UPLC/ESI/TOFMS to determine nitrogen fluxes

Annika I. Johansson, Camila Aguetoni Cambui, Cathrine Campbell, Vaughan Hurry, Torgny Näsholm, Thomas Moritz

Understanding nitrogen uptake and internal nitrogen fluxes in a tree is economically important, since nitrogen (N) is the key determinant in tree growth. By studying the uptake and assimilation of 15N-labelled ammonium and nitrate in poplar trees the uptake routes and the continuous flux in the amino acid metabolism can be understood.

In this study we show a feeding experiment, where poplar plants were fed with 15N-labelled ammonium and nitrate. For the analysis of this experiment we have developed a protocol for analysis of AccQ-TagTM derivatised amino acids by UPLC/ESI/TOFMS.

Populus trees were grown in liquid culture, and after a short starvation period the trees were fed with 15N-labelled ammonium and nitrate. Samples (roots and leaves) were taken after 0, 0.5, 1, 3 hours and a long effect sample after 7 days. The amino acids were extracted using a methanol:water:chloroform (3:1:1) mixture. The dried extract was dissolved in 10mM HCl and derivatised using the AccQ-TagTM (Waters). The derivatized amino acids were analyzed on UPLC/ESI/TOFMS. The accumulated data were integrated and isotope ratios calculated to follow the incorporation of 15N.

By derivatizing the amino acids with AccQ-TagTM the chromatography and sensitivity of the amino acids increased dramatically compared to its non-derivatized form. The AccQ-TagTM derivatization was developed for HPLC/UV-analysis in the beginning of the 1990’s. The method has proven to be a robust and sensitive method for amino acid analysis. Interestingly we found that the sensitivity of the amino acid derivatives is even better on our ESI/TOFMS. Another advantage by using mass spectrometry is a more reliable identification of the analytes and this is especially useful when unclear peaks are found in the chromatogram. By using mass spectrometry we are able to follow the incorporation of 15N by studying the isotopic pattern of the derivatised amino acids. Preliminary results from our feeding study shows incorporation of 15N in most of the studied amino acids in both roots and leaves. The incorporation seems to be faster in the roots compared to the leaves. The rate of incorporation also differs between the different amino acids. Using this data our aim is to present a “road map” of nitrogen uptake and internal nitrogen fluxes in Populus.
Organic nitrogen is transported in the form of amino acids, that's why the amino acids are considered as the “currency of nitrogen exchange” in the plants. Recent studies of *Arabidopsis thaliana* (Arabidopsis) have identified three amino acid transporters involved in root amino acid uptake: Lysine Histidine Transporter 1 (LHT1), Amino Acid Permease 1 (AAP1) and Amino Acid Permease 5 (AAP5). In this study we used Arabidopsis plants with altered expression of LHT1, AAP1 and AAP5 with the aim to disentangle the role of each transporter for uptake of different amino acids at naturally-occurring (2-50 μM) concentrations.
Sabine Kunz

**Studying the mechanism of sucrose specific gene expression in A.thaliana cell culture Presenting author**

Sabine Kunz

**All authors**

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**Abstract**

The ability of a plant to cope with different environmental factors depends on its energy state. In plants, the energy available for each cell underlies a continuous fluctuation, which is influenced by carbon assimilation and respiration. The reaction of a plant cell on available carbohydrates (CH), an important source of energy, is partly coordinated through cell signaling, especially in the aspect of specific changes in gene expression. Those are determined by the interaction of a transcription factor (TF) with cis-regulatory DNA elements in promoters of responsive genes. Here we describe experimental approaches to identify and verify the functionality of predicted cis-elements; which are involved in sucrose-dependent cell signaling. The use of A. thaliana cell culture, grown on xylose as carbon source, allows us to distinguish the response of the cells to different CH signals. This set-up, applied to cells transformed with promoter-reporter gene constructs that bear the cis-elements in both the forward and reverse orientation in a native as well as synthetic environment, provides us with hints for the involvement of the cis-elements in the different transcriptional responses.
Salma Chaabouni

Unravelling the role of Aux/IAA proteins on adventitious rooting in Arabidopsis: Identification of ARF6 and ARF8 partners

The plant hormone auxin has emerged as a central coordinator of plant growth. It regulates embryonic and post-embryonic root initiation and development and plays a key role in the control of adventitious rooting (AR). The ability to clonally propagate a broad range of tree species is hampered by the inability to induce AR in the propagules, therefore understanding AR development is of prime importance to capture and maintain elite plant characteristics. Recent findings from our group showed that AR, in the model plant Arabidopsis, is controlled by a complex network of AUXIN RESPONSE FACTORS (ARF) and microRNAs. ARF17, a target of miR160, is a negative regulator of AR while ARF6 and ARF8, targets of miR167, are positive regulators. Aux/IAA proteins are known to be interacting partners of ARFs. Many have been shown to be negative regulators of auxin-induced transcription through their interaction with ARF proteins. We hypothesize that in absence of auxin, the transcriptional activity ARF6 and ARF8 in the regulation of AR is repressed by at least one of the Aux/IAA members. We propose that endogenous auxin or exogenously applied auxin would release the inhibition of ARF6 and ARF8 by inducing the degradation of one or more Aux/IAAs and in this way lead to the induction of GH3 genes and the promotion of adventitious root formation. Using a combinatory approach (Analysis of mutants, qRT-PCR technology and in vivo coimmunoprecipitation), the identification of Aux/IAA(s) that potentially interact with ARF6 and ARF8 in the AR regulatory pathway will be discussed.
Screening and characterization of superroot2-1 suppressors (srs)

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Adventitious root formation allows clonal propagation and rapid fixation of superior genotypes prior to their introduction into production or breeding programs. This strategy is often used for long-lived woody species. Nevertheless the inability to initiate adventitious roots remains an obstacle for elite genotypes of many crop and woody species and the regulatory mechanisms are still not well understood. The hormone auxin is one of the main endogenous factors controlling adventitious root formation and the superroot (sur1 and 2) mutants over-produce auxin and spontaneously make adventitious roots. In an attempt to identify new genes involved in the regulation of adventitious rooting we performed a screen for suppressors of the sur2 mutation. 2345 independent M2 families derived from sur2-1 homozygote seeds mutagenized with EMS were screened for the suppression of the adventitious root phenotype. 45 suppressors were confirmed in the M3 progeny, and complementation crosses indicate that they belong to 38 groups of complementation. F2 mapping populations after crossing with atr4-1, a sur2 allele in Col-0 background, have been produced for all the suppressors. A more detailed characterization was performed for 24 suppressors. Their position on the chromosomes has been established and the map based cloning of the suppressor genes is in progress. For selected mutants, the free and conjugated IAA levels were quantified. In suppressors sar1 and sar2 the total auxin content is back to the WT level, while in the suppressors sar3, sar4, sar5 and sar6 the free IAA level is similar as in sur2-1. These preliminary data show that these mutants will allow in the future to identify new genes that not only regulate adventitious root formation but most likely also auxin homeostasis.
Amir Mahboubi

**Identifying molecular sugar responses in Arabidopsis**

Amir Mahboubi, Concetta Valerio and Totte Niittylä Department of Forest Genetics and Plant Physiology, UPSC, Umeå.

Growth and development of a plant requires precise control and coordination mechanisms of sugar metabolism. The aim of this project is to understand how plants respond to changes in sugar levels at the molecular level. In our first approach Arabidopsis seedlings were grown in liquid medium with sucrose and then depleted of carbon before sucrose was resupplied. Soluble proteome derived phosphopeptides were analyzed quantitatively by mass spectrometry over five time points of sucrose resupply. This analysis identified a protein we named Sucrose C-terminus Dephosphorylated (SCDP). A carboxyterminal serine of SDP was rapidly dephosphorylated in response to sucrose and the same serine had previously been shown to be phosphorylated in response to ethylene. Null mutants of scdp showed a stunted growth phenotype. In our second approach we have used forward genetics to screen for suppressors of cob-2. cob-2 has an amino acid change in the COBRA protein, which is essential for cellulose biosynthesis. cob-2 is a conditional mutant showing a root swelling phenotype only in the presence of exogenous sugars. The mechanism of this sugar inducible phenotype is not known but may be linked to regulation of sugar homeostasis and sugar flux to cell walls. We screened an EMS mutagenised population of cob-2 and identified 8 mutants in which the sugar response was suppressed.
Concetta Valerio

Unravelling carbon allocation to woody biomass in aspen

Concetta Valerio, Amir Mahboubi, Melissa Roach, Björn Sundberg, Mattias Hedenstrom and Totte Niittylä.

The aim of this project is to develop new tools to quantify carbon flux from photosynthetic tissues to wood biosynthesis and to identify the transport mechanisms and enzymatic steps required for sucrose transport from phloem to developing wood. Determining the rate and route of carbon flux to wood has fundamental value for understanding tree biology. We are using following approaches: (1) Aspen fluxomics: we labelled 6-weeks-old wild-type aspen trees using a 2-hour 13CO2 pulse after which the label accumulation in the developing wood was followed for 2, 4 and 6 hours. Using two-dimensional NMR we established that 2 hours after the 13CO2 pulse strong enrichment of the 13C label could already be detected in the developing wood. Further analysis should allow the label distribution and the rate of accumulation in different wood polymers (cellulose, hemicellulose, lignin) to be determined. (2) The ray transcriptome: Wood ray cells represent a likely lateral carbon transport route, to better understand ray cell function we isolated RNA from ray cells using laser microdissection. The RNA is being sequenced alongside the wood developmental gradient samples to provide a spatial understanding of ray cell specific transcripts. (3) Micro-scale analysis of metabolites and enzyme activities across the tangential gradient of wood development. This high-resolution profile will ultimately reveal the soluble carbohydrate status and associated enzyme activities across the phloem, cambium, xylem differentiation and expansion, and secondary wall deposition zones of the developing wood. This analysis will serve to identify key enzyme activities associated with each stage of development, and thus pinpoint potential routes of carbon flux to wood cell walls.
Marcia Rosa

Dissecting sterol function during cytokinesis and clathrin-dependent endocytosis in Arabidopsis

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Sterols are lipid components of plant membranes necessary for membrane sorting and trafficking processes (1). In mammalian cells and yeast, alteration of the amount of free sterols leads to defects in the endocytic pathway. The internalization of various plasma membrane (PM) proteins in animal cells engages clathrin-coated vesicles. Yet, comparatively little is known about clathrin-dependent endocytosis and its role during plant cytokinesis. The isolation of the sterol biosynthesis mutant cyclopropylsterol isomerase 1-1 (cpi1-1) in Arabidopsis, has provided a valuable tool for further understanding the role of plant sterols during endocytosis [2-4]. The cpi1-1 mutant displays a strong conversion of the sterol profile and almost exclusively accumulates cyclopropyl sterols on expense of the wild-type membrane sterols [2]. In this mutant, a mediator of cytkinetic vesicle fusion in the cell division plane, the syntaxin KNOLLE, is no longer constricted to the division plane but mis-localized to the lateral membrane upon fusion of the cell plate with the PM in late cytokinetic cells [3]. Treatment of wild type and cpi1-1 mutant roots with the endocytic recycling inhibitor brefeldin A revealed that KNOLLE internalization into endosomal membrane agglomerations compared to its level at the cell division plane and plasma membrane is affected in the cpi1-1 mutant. These findings suggested that KNOLLE is constrained to the cell division plane at the end of cytokinesis through sterol-dependent endocytosis. Consistent with this view, pharmacological interference with internalization mediated through clathrin-coated vesicles induced KNOLLE mis-localization at lateral membranes in late cytokinetic cells. Similarly, in dynamin-related protein 1a (drp1a) mutants defective in a component associated with the clathrin machinery and affected in endocytosis, KNOLLE displayed lateral mis-localization. Therefore, KNOLLE appears to be constrained to the cell division plane at the end of cytokinesis through a sterol-dependent endocytosis mediated by clathrin and DRP1A [4]. Here, we present further biochemical evidence supporting that KNOLLE is affected in the cpi1-1 mutant and also that components of the clathrin mediated endocytosis are preferentially found in sterol-enriched membrane fractions. For this purpose, we carry out analysis of detergent-resistant membrane (DRMs) fractions. In addition we have performed immunolocalization experiments of DRP1A in roots of both wild type and the cpi1-1 mutant and initiated clonal loss-of-function mosaic analyses of the CPI1 gene.

Hailiang Mao

Differential functions of the domains of the GS3 protein in regulating grain size in rice

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Abstract Grain yield in many cereal crops is largely determined by grain size. Here we report the genetic and molecular characterization of GS3, a major QTL for grain size. It functions as a negative regulator of grain size and organ size. The wild type isoform is composed of four putative domains: a plant specific OSR domain in the N-terminus, a transmembrane domain, a TNFR/NGFR family cysteine-rich domain, and a VWFC in the C-terminus. These domains function differentially in grain size regulation. The OSR domain is both necessary and sufficient for functioning as a negative regulator. The wild type allele corresponds to medium grain. Loss-of-function of OSR results in long grain. The C-terminal TNFR/NGFR and VWFC domains show inhibitory effect on the OSR function; loss-of-function mutations of these domains produced very short grain. This study linked the functional domains of the GS3 protein to natural variation of grain size in rice.
Jebasingh Selvanayagam

Cracking the code of bZIP dimerization in Arabidopsis

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S1 group Basic Leucine Zipper Domain (bZIP domain) transcription factors (bZIP1, bZIP2, bZIP11, bZIP44, bZIP53) form heterodimers with C group bZIP transcription factors (bZIP9, bZIP10, bZIP25, bZIP53). The dimers bind to ACGT core motives which have been identified in a multitude of plant genes regulated by diverse environmental, physiological and environmental cues. Specific S1/C dimer formation has been demonstrated using both Yeast two hybrid and Plant two hybrid analysis, but physiological relevance remained unclear. Our microarray analysis shows that different dimers regulate different set of genes in a specific manner. Interestingly, bZIP11 is affecting gene expression significantly more than other tested bZIP proteins both when over expressed alone or in combination with dimerizing partners. However, dimerization is in all cases enhancing activity of all bZIPs. Moreover, using gain and loss of function mutants reveal the importance of bZIPs dimer regulation in plants. Based on this, our proposed model suggests that the combinatorial control of amino acid metabolism and regulation of stress target genes are controlled by a network of specific heterodimers of bZIP transcription factors. This network gives the plant ample opportunity to fine-tune responses to a variety of factors by modulating the individual genes of bZIP proteins in the cell.
Douglas G. Scofield

Comparative variation of the chloroplast in four Populus species through next gen sequencing and de novo assemblies

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The use of next-generation sequencing technology allows for an unprecedented opportunity to examine inter- and intraspecific evolution of the chloroplast genome. Using the Illumina GAII platform, we sequenced a pooled set of 20 trees per species from throughout the ranges of Populus trichocarpa, P. balsamifera, P. fremontii and P. deltoides. Whole-chloroplast genomes were assembled de novo for each species and compared to evaluate colinearity, detect structural rearrangements, and examine the diversity and divergence across chloroplast genes. There have been few structural changes to the chloroplast genome within each species. We found about 1500 indels of 1-15 bp and approximately 1500 SNPs across all 80 trees. A total of 468 SNPs were identified that differentiated P. balsamifera, P. deltoides or P. fremontii from the P. trichocarpa reference genome. Nearly all indels and two-thirds of SNPs occur outside of RNA- and protein-coding genes, and no SNPs occurred within RNA-coding genes. Within protein-coding genes, frequencies of synonymous and non-synonymous SNPs are approximately equal in all four species, with non-synonymous changes occurring in a variety of chloroplast genes including those coding for members of photosystems I and II, the ndh complex, and RNA polymerase. Future work will include the addition of chloroplast genomes for P. tremula, as well as a broader examination of evolutionary signatures within different functional classes of genes along with environmental data from the provenances of origin.
Hanna Johansson Jänkänpää

A retrograde signal originating at PSII influence herbivore preferences

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Under natural conditions, plants have to cope with a multitude of stresses, two of those are light-stress and herbivory. Plants have evolved several mechanisms to avoid the damage done by strong and fluctuating light. One of the most efficient photoprotection mechanism is the qE-type of non-photochemical quenching (NPQ), a process where the protein PsbS is highly involved. Light-stress causes a variety of changes in the metabolism of plants and these changes also influence plant–herbivore interactions.

To test the effect on herbivores of different levels of photoprotection, we used Arabidopsis thaliana wild-type and two photoprotection genotypes, npq4 and oePsbS that, respectively, lack and overexpress the PsbS protein. Food choice and oviposition preference experiments were conducted with a specialist and a generalist insect herbivore. We have also studied metabolomics of the npq4 and oePsbS plants when transferred to the field, where the plants experienced both light and herbivore stress.

In dual-choice feeding experiments, both insect herbivores preferred the less light stressed plants. For oviposition, female adults of diamondback moth were preferentially attracted to more light stressed plants. Metabolomics result point to a weather dependent shift between the genotypes. This together with a study of reactive oxygen species (ROS) and marker genes of the jasmonic acid and salisylic acid pathways result in a model where superoxides released at photosystem II (PSII) influence herbivore preferences.

Thus, in Arabidopsis plants, the variation in the abundance of a single protein (PsbS) that affects the regulation of light harvest, influenced preferences and the performance of insect herbivores. Our results suggest that, in plants, there may be opposing selection pressures on their capacity to handle both light-stress and herbivory.
Functional Characterization of a Microtubule-Associated Protein, MAP20, in *Populus*

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Plant cortical microtubules (MTs), which are self-organizing, are thought to provide a guidance mechanism for the ordered deposition and alignment of cellulose microfibrils (MFs) via a host of different MT-regulatory proteins, collectively known as microtubule-associated proteins (MAPs). Recently, Rajangam et al. (2008) described a MAP in *Populus*, PttMAP20, which binds to taxol-stabilized microtubules in vitro. This gene is highly expressed during the formation of secondary cell walls in *Populus* wood fibres and co-regulates with secondary cell wall associated cellulose synthase genes (*CESAs*). We are carrying out functional analysis of PttMAP20 and its closest Arabidopsis homolog, AtMAP20, in order to establish the putative linkage to secondary cell wall biosynthesis.

Over-expression of both the *Populus* and the Arabidopsis MAP20 gene in Arabidopsis seedlings leads to right-handed helical twisting of epidermal cell layers in roots and hypocotyls, an observation that is in agreement with previously described MAPs. Promoter–GUS analysis of PtMAP20::EGFP-GUS lines (2 kb *Populus MAP20* promoter) shows expression in developing xylem cells, predominantly at the early stage of secondary cell wall formation. In addition, we have produced transgenic *Populus* RNAi lines, as well as 35S::PttMAP20 over-expression (OE) lines. No obvious growth phenotype was observed, though. These transgenic lines are currently being phenotyped for wood characteristics using a battery of FuncFiber wood chemistry tools to unravel gene function during xylogenesis.

Data obtained from FT-IR fingerprinting indicates that both RNAi and OE lines have a different chemotype compared to wild type, which is more pronounced in young developing secondary xylem tissues. OPLS-DA analysis suggests that the relative amount of lignin and cellulose are proportionally higher, while hemicelluloses are proportionally lower, in transgenes. Further, chemical analysis of cell wall monosaccharides released after TFA hydrolysis demonstrates a relative higher amount of rhamnose and lower amount of glucose in transgenes. In addition, data from soluble cytosolic sugars implies a higher content of sucrose in transgenes. Interestingly, the crystalline cellulose content, as determined by the Updegraff method, was unaffected. Furthermore, a chemical phenotype is also demonstrated by Py-GC/MS analysis, confirming a significantly higher amount of lignin in PtMAP20-OE. Preliminary mechanical analysis suggests a lower stiffness in wood of PtMAP20-OE, implicating a higher microfibril angle (MFA), an important wood quality trait. Microarray data of transcripts from developing xylem of RNAi lines reveal significant mis-regulation in a number of genes involved in carbohydrate metabolism along with some recognized transcription factors. In addition, the altered expression of some signalling, transport and cell wall related genes was obvious.

Finally, MAP20 is a small cytosolic protein containing a TPX2 domain, a domain originally described in *Xenopus* as a target for a kinesin-like protein. When deletion constructs of PtMAP20 were transiently expressed in Tobacco leaf cells, we discovered that the N-terminal and TPX2 region are probably required for microtubule binding, since this construct decorated microtubules in a convincing way, as is demonstrated by confocal microscopy studies.