20th International Conference on Arabidopsis Research

Edinburgh, Scotland, United Kingdom

30th June - 4th July 2009

Conference Abstracts

Contents

Genome Organisation

Development

Environmental Responses

Plant Defence

Natural Variation

Cell Biology

Systems Biology

Hot Topics

New Tools and Resources

Plant Growth Receptors

Bioenergy

Peroxisome

Posters

Genome Organisation

P001 Arabidopsis whirly proteins maintain genomic stability in plastids

Jean-Sebastien Parent, Alexandre Marechal, B Franz Lang, Normand Brisson

Department of Biochemistry, Universite de Montreal, PO Box 6128, Station Centre-ville, Montreal, Quebec, Canada, H3C 3J7

Maintenance of genome stability is essential for the accurate propagation of genetic information as well as for cell growth and survival. Plants have the particularity of possessing three different genomes that require such maintenance. While many factors involved in the protection and repair of the nuclear DNA have been reported, surprisingly little is known about these phenomena in plant organelles. The Whirlies form a highly conserved protein family found mostly inside the plant kingdom. Their ubiquitous presence in plant organelles and their capacity to bind single-stranded DNA with little specificity make them ideal candidates for the protection of plastid genomes. We show here that Arabidopsis plants lacking plastidial Whirlies can develop variegated tissues in some individuals. This trait is maternally inherited which is indicative of the organellar nature of the defect. Indeed, the phenotype was linked to an increase in illegitimate recombination between short direct repeats found in the plastids of all mutant plants. In rare cases this type of recombination was also found in wild-type plants. We propose that the Whirlies maintain the genomic stability in plastids by preventing micro-homology mediated recombination.

P002 Manipulating meiosis: Crossovers from Arabidopsis to crops

Claire Halpin, Abdellah Barakate, Robbie Waugh, Luke Ramsay, Glyn Jenkins, Dylan Phillips, Sue Armstrong, James Higgins, Chris Franklin

Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK

Meiotic recombination is one of the principal forces creating the genetic diversity that drives evolution and is the fundamental instrument underlying most crop breeding programs. A greater understanding of the control of recombination in crops would enable manipulation of this process to improve the speed and accuracy of plant breeding. Over the past decade, studies in the model plant Arabidopsis have resulted in considerable progress in understanding how meiosis and recombination in plants is controlled at the molecular level. The development of novel molecular cytogenetic techniques and reagents has underpinned the use of forward and reverse genetic approaches to determining gene function. This has led to the characterisation of a growing number of genes involved in meiotic recombination and has revealed the existence in Arabidopsis of more than one pathway to crossovers. Understanding how the frequency and distribution of meiotic crossovers are controlled would be particularly useful for some of the UK's most important crop species (wheat, barley, oats, and forage grasses) where the distribution of crossover events is highly skewed. Chiasmata appear to be preferentially targeted to sub-telomeric regions of the chromosomes. In contrast centromere proximal regions rarely recombine, even though they contain substantial numbers of genes. Our objectives, within this BBSRC-funded LOLA project, are to take the knowledge and techniques used to elucidate meiosis in Arabidopsis and use them to determine how meiotic recombination is controlled in barley in order to illuminate the basis for the skewed pattern of recombination. We will then explore strategies that could be used to manipulate the patterns of recombination to improve future crop breeding.

P003 An orthologous transcriptional signature differentiates responses among closely related chemicals in *Arabidopsis thaliana* and *Brassica napus*

Malay Das1,2, Georg Haberer3, Jay R Reichman1, Gerhard Welzl3, Felipe F Aceituno4, Michael T Mader3, Lidia S Watrud1, Tom G Pfleeger1, Rodrigo A Gutierrez4, David M Olszyk1, Anton R Schaffner3

1U.S. Environmental Protection Agency, Western Ecology Division, Corvallis, Oregon, USA, 2Present address: Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA, 3Helmholtz Zentrum Munchen, Neuherberg, Germany, 4Pontificia Universidad Catulica de Chile, Santiago, Chile

Herbicides are structurally diverse chemicals that inhibit plant-specific targets, however their off-target and potentially differentiating side-effects are less well defined. In this study, genome-wide expression profiling based on Affymetrix ATH1 arrays was used to identify discriminating responses of *A. thaliana* to five related chemicals that target two different branches of amino acid biosynthesis. The compounds included glyphosate that targets 5-enolpyruvylshikimate-3-phosphate synthase and four diverse acetolactate synthase (ALS) inhibiting compounds. The ALS inhibitors represented three different chemical classes and included two compounds based on a sulfonylurea backbone. In contrast to glyphosate, which affected only a few transcripts, ALS inhibitors led to numerous transcriptional changes which indicated off-target effects related to C-metabolism, secondary metabolism, cell wall modification and growth. The expression pattern of a set of 101 genes provided a specific, composite signature that was distinct from other major stress

responses and differentiated between chemicals targeting the same enzyme (ALS) and even between those possessing the same structural backbone (sulfonylurea). Despite the complications due to a genome triplication event during evolution of Brassica species, a set of homologous genes was identified in *Brassica napus* that also exhibited a similar expression pattern distinguishing the exposure to the five herbicides. Hence, the ability of a limited number of orthologous genes to classify and differentiate responses to closely related chemicals in *A. thaliana* and *B. napus* underscores the validity of such a feature and the transferability of a complex transcriptional signature across species.

P004 Investigating novel potential regulators and signalling components in phosphate stress responses of *Arabidopsis thaliana*

Magdalena Musialak-Lange1, Rosa Morcuende1, Wolf-Ruediger Scheible2

1Max Planck Institute for Molecular Plant Physiology, Potsdam/Golm, 2Instituto de Recursos Naturales y Agrobiología de Salamanca, CSIC

Bioinformatic analysis of expressed sequence tags in Arabidopsis has led to the identification of “orphan RNA transcripts” (oRNAs) which contain either no or only very short open reading frames. These oRNAs could code for small peptides or small functional RNAs with so far unknown role. Accumulating evidence however indicates that "non-coding RNAs" in various species play crucial roles in a range of cellular processes, including epigenetic silencing, transcriptional regulation, developmental control and stress related responses.

Since oRNAs are not represented on public gene expression platforms (e.g. Affymetrix GeneChips) we established a sensitive qRT-PCR platform to analyse the expression and induction of nearly 600 Arabidopsis orphans in various conditions. We showed that expression of some of the RNA transcripts is strongly and specifically changed under Pi (and other nutrient) starvation conditions. In addition, the constitutive up-regulation of one of the Pi responding oRNAs leads to a significant increase of starch levels in plants. Together with the finding, that this oRNA was highly abundant in the polisomal fraction of RNA, we suggest a role of this oRNA in catalytical processes controlling the carbohydrate status in plants in relation to Pi levels. This is supported by the recent discovery that a microRNA, which is regulated by non-coding RNA (2), is involved in Pi stress related responses.

Our data suggest that other oRNAs could also be involved in the modification/ regulation of the Pi response. The ongoing study of these oRNAs, including reverse genetic approaches and an analysis of the putative orphan promoters, should contribute to our understanding of the complex network of metabolite signalling in plants.

P005 Generation and transcriptome analysis of autotetraploid *Arabidopsis thaliana*

Zheng Yu1, Kristina Haage1,2, Michaela Matthes1, Verena Streit1, Georg Haberer3, F X Klaus Mayer3, Alfons Gierl1, Ramon A Torres-Ruiz1

1Lehrstuhl fur Genetik, TU Munchen, Freising, Germany, 2Bereich Genetik, LMU Munchen, Planegg-Martinsried, Germany, 3MIPS/IBI, HelmholtzZentrum, Neuherberg, Germany

At the beginning of their “life”, newly occurring polyploids have to cope immediately with the selection pressure imposed by the environment and their (competing) diploid progenitors. Modulation of gene expression programs is thought to be the key element of neo-polyploid plants for successful adaptation. Allopolyploid plants exhibit extensive alterations of gene expression, probably caused by conflicts of divergent genomes. In contrast, autopolyploid plants have multiplied uniform genomes. Information on gene expression alterations of this form of polyploidy is limited even when Arabidopsis is considered, since only few autotetraploid *Arabidopsis thaliana* lines are available. We have therefore developed a novel strategy for the rapid generation and identification of polyploids based on trichome branching patterns and analyzed over two dozens independently induced *Arabidopsis thaliana* autotetraploids. We have obtained comprehensive information on the relationship between cell size and ploidy levels and on the relative stability of tetraploidy. The neotetraploid lines exhibit considerable stability through at least three consecutive generations. We have subjected numerous *Arabidopsis thaliana* autotetraploids to transcriptome analyses, which reveal that changes in gene expression strongly depend on the genotype. We are analysing these lines with respect to transcriptome pattern of different ploidies, ecotypes, tissues and generations. We discuss our results with respect to the impact of natural variation on autopolyploid gene expression and on the evolution of autopolyploid plants.

P006 Intron retention in Arabidopsis mRNA transcripts

Craig G Simpson1, Maria Kalyna2, John Fuller1, Diane Davidson1, Andrea Barta2, John WS Brown1,3

1Genetics Programme, SCRI, Invergowrie, Dundee, DD2 5DA, UK, 2Max F. Perutz Laboratories, Medical University of Vienna, Institute for Medical Biochemistry, DR. Bohr-Gasse 9/3, 1030 Vienna, Austria, 3Division of Plant Sciences, University of Dundee, Dundee DD2 5DA

Intron retention (IR) is the most commonly described alternative splicing (AS) event in plants. Around 40% of all described plant AS events show intron retention compared to ~10% in the human genome (1). Evidence shows that some plant mRNAs that retain introns are assembled onto ribosomes (2). Nevertheless, it is not clear how many retained introns reflect the cloning of partially spliced introns as ESTs, result from inefficient splicing, or are conserved, functionally relevant and regulated AS events. We have analysed in detail 46 recognised AS events using an RT-PCR based panel of primers that

allows us to monitor changes in the relative abundance of the different alternatively spliced transcripts. Primer pairs were designed to amplify gene regions that span the retained intron and 2-4 efficiently spliced introns. Using these primers, we were able to determine to what extent AS isoforms with retained introns contribute to the total AS transcript profile for each gene. Analysis of plants grown under different conditions and in mutants involved in different aspects of mRNA processing showed that around half of the selected IR events were undetectable. This suggests that these mRNAs are rare, probably represent partially spliced transcripts in EST databases and are unlikely to be real alternative splicing events. Only 6 IR events showed IR transcripts that contributed >10% of the total AS transcripts, which included two with in-frame introns. This group of IR events may have important functional roles in post-transcriptional regulation of their cognate genes. Finally, IR usually results in the presence of premature termination codons (PTCs). Using mutants in the nonsense mediated decay (NMD) pathway, none of the 13 detectable IR transcripts which introduce PTCs showed evidence of turnover by NMD, indicating that IR transcripts may not be targets of NMD.

Barbazuk *et al* (2008) Genome Research 18: 1381-1392; 2. Ner-Gaon *et al* (2004) Plant J. 39: 877-885.

P007 Arabidopsis PTB-like 1 (AtPTBL1) negatively regulates splicing inclusion of a plant mini-exon

Craig G Simpson1, Sean Chapman2, Michele Liney1, Diane Davidson1, Dominika Lewandowska1, John WS Brown1,3

1Genetics Programme, SCRI, Invergowrie, Dundee, DD2 5DA, UK, 2Plant Pathology Programme, SCRI, Invergowrie, Dundee, DD2 5DA, UK, 3Division of Plant Sciences, University of Dundee @ SCRI, Invergowrie, Dundee, DD2 5DA, UK

Alternative splicing is regulated by the interactions of splicing enhancer and suppressor proteins with sequence signals on the precursor mRNA. Polypyrimidine tract binding protein (PTB) in animals is an RNA-binding protein which represses splicing of many alternatively spliced exons by binding to CU-rich sequences. PTB suppresses splicing by competing with U2AF65 for the polypyrimidine tract, blocking access of splicing factors to splicing signal or enhancer sequences, causing looping out of branchpoint or exon sequences or interfering with interactions needed for intron or exon definition or spliceosome assembly (Spellman and Smith, 2006).

We have examined whether a putative Arabidopsis orthologue of PTB (AtPTBL1) is a splicing repressor using viral expression and protoplast transient expression systems with a mini-exon splicing reporter system. The mini-exon reporter system is based on splicing of the potato invertase mini-exon which requires strong constitutive signals in the upstream intron to drive inclusion of the 9 nt exon. Inclusion of the mini-exon is very sensitive to weakening of the splicing signals, which leads to skipping of the mini-exon. We find that AtPTBL1 reduces inclusion/splicing of the mini-exon. Mutation of pyrimidine-rich sequences in the 3' half of the intron upstream of the mini-exon showed that repression of mini-exon splicing by AtPTBL1 required the polypyrimidine tract and a downstream CU-rich sequence. Co-expression of the SR protein, AtU2AF65, in polypyrimidine tract mutants alleviated the splicing repression of AtPTBL1 suggesting that these proteins compete for binding to the polypyrimidine sequences, and that, therefore, AtPTBL1 functions in splicing repression.

Spellman, R & Smith, CW. 2006. Trends Biochem Sci 31, 73-76

Development

P008 Dynamic changes of histone H3K27 tri-methylation during plant development

Marcel Lafos, Phillip Kroll, Daniel Schubert

Heinrich-Heine University, Duesseldorf, Germany

Many processes during plant and animal development are epigenetically regulated. The expression of key regulators of flowering time *(FLOWERING LOCUS C)*, flower *(AGAMOUS)* and seed development (*MEDEA)* is affected by changes in histone methylation. Polycomb-group (Pc-G) proteins were shown to regulate histone H3 lysine 27 trimethylation (H3K27me3). Loss of PcG-function correlates with the de-repression of target genes causing aberrant developmental programs and changes in cell identity (e.g. floral reversion). Phenotypes of PcG mutants imply that cell fate is not maintained suggesting a reversible regulation of cell identity by Pc-G and H3K27me3.

Our genome wide analysis of H3K27me3 in different tissues uncovered novel target genes not detected in former studies based on the analysis of whole seedlings. The comparison of these data sets has revealed genes that were exclusively covered with repressive histone methylation (H3K27me3) in differentiated (leaf) but not in undifferentiated (meristem) tissue or vice versa. The observation of differentially methylated genes supports the idea of highly dynamic changes in chromatin state during differentiation and plant development. A negative correlation between H3K27me3 coverage and gene expression was confirmed by our analysis as well.

P009 Signaling triggered by activation of CC-NB-LRR-related UNI affects SAM activity in a non-cell-autonomous manner involving ERECTA receptor kinase

Naoyuki Uchida, Kadunari Igari, Masao Tasaka

Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan

The shoot apical meristem (SAM) of plants is an indeterminate structure and the source of stem cells from which all post-embryonic aerial organs are derived. The regulatory network which functions within the SAM to control its activity has been well studied and some reports suggested that the signals for the SAM regulation are provided also from outside of the SAM. However, the

molecular mechanisms, which are involved in the regulation of such signals, are largely unknown. We previously isolated the Arabidopsis *uni-1D* mutant harboring a semidominant and gain-of-function allele of the *UNI* gene, which has a structure related to the CC-NB-LRR family, and interestingly *uni-1D* plants show morphological phenotypes; a defect of SAM maintenance and ectopic formation of axillary meristems. Here we show that the activation of *UNI* outside the SAM non-cell-autonomously attenuates the SAM activity, which is accompanied by remarkable reduction of the expression of the *WUSCHEL* gene, a key player controlling stem cell population within the SAM. We also show that the function of ERECTA (ER) receptor kinase within the UNI-expressing region outside the SAM is required for the SAM defect of *uni-1D mutants* but not for the other *uni-1D* phenotypes, indicating that ER coordinates only a part of signal transduction pathways triggered by activation of *UNI*. Taken together, our findings suggest novel aspects of the molecular mechanisms for the SAM regulation.

P010 The trihelix transcription factor AtGTL1 controls ploidy-dependent cell growth in the Arabidopsis trichome

Christian Breuer1, Ayako Kawamura1, Takanari Ichikawa2, Rumi Tominga-Wada3, Takuji Wada3, Youichi Kondou1, Shu Muto4, Minami Matsui1, Keiko Sugimoto1

1RIKEN Plant Science Center, Yokohama, Kanagawa, Japan, 2University of Tsukuba, Tsukuba, Ibaraki, Japan, 3National Institute for Basic Biology, Okazaki, Aichi, Japan, 4Valway Technology Center, NEC Soft Co. Ltd., Tokyo, Japan

Leaf trichomes in Arabidopsis develop through several distinct cellular processes such as patterning, differentiation and growth. Although recent studies have identified several key transcription factors controlling early patterning and differentiation steps, it is still largely unknown how these regulatory proteins interfere with subsequent trichome development accompanied by rapid cell growth and branching. Here we report a new trichome mutation, which in contrast to all other previously identified mutants, affects only trichome cell growth without altering its overall patterning or morphology. We show that the corresponding gene encodes an AtGT-2-LIKE1 (AtGTL1*)* protein, a member of the trihelix transcription factor family. Loss of *AtGTL1* function leads to a dramatic increase in trichome cell size and ploidy, suggesting that AtGTL1 is a negative regulator of ploidy-dependent cell growth in trichomes. The *pAtGTL1:GUS* and *pAtGTL1:AtGTL1:GFP* fusion reporter lines reveal that AtGTL1 is present within the nucleus during post-branching stages of trichome development. Consistently, our genetic analyses show that *AtGTL1* acts downstream of early differentiation genes through a novel genetic pathway that is independent from previously described *GL3* or *TRY* mediated pathways. Our data strongly suggest that AtGTL1 is part of the novel regulatory networks that link early trichome differentiation to its final development. This study also provides the first genetic evidence for the requirement of transcriptional controls in repressing plant cell growth as well as for an involvement of GT-2-like proteins in this control.

P011 Proliferation and cell fate establishment during Arabidopsis male gametogenesis depends on Retinoblastoma

Zhong Chen1, Said Hafidh2, Shi Hui Poh1, David Twell2, Frederic Berger1

1Temasek Life Sciences Laboratory, Singapore, 2Department of Biology, University of Leicester, UK

Retinoblastoma (Rb) is a conserved repressor of cell proliferation. In animals Rb deregulation also perturbs cell differentiation. In plants reduced levels of Rb cause hyperproliferation and impair differentiation to various degrees in leaves, roots and female gametes. However the primary developmental impact of the loss of Rb has remained unclear. Here we investigated the direct consequences of Rb knock-out in the plant male germline using cytological and molecular markers. The plant germ line is produced by unequal division of the microspore into a small germ cell and a large terminally differentiated vegetative cell. A single division of the germ cell produces the two sperm cells, which no longer divide. We observed that the loss of Rb does not have a major impact on microspore division but causes limited hyperproliferation of the vegetative cell and to a lesser degree of the sperm cells. In addition cell fate is perturbed in a fraction of Rb-defective vegetative cells. These defects are rescued by preventing cell proliferation arising from down-regulation of the cycle dependent kinase CDKA1. Our results indicate that hyper-proliferation caused by the loss of Rb prevents or delays cell determination during plant male gametogenesis, providing further evidence for a direct link between fate determination and cell proliferation.

P012 Novel MAG2-interacting proteins are involved in vacuolar sorting of seed storage proteins

Lixin Li1,2, Tomoo Shimada2, Hideyuki Takahashi2, Baoyu Tu1, Hongmin Jin1, Baoda Han1, Junpei Takagi2, Maki Kondo3, Mikio Nishimura3, Ikuko Hara-Nishimura2

1College of Life Science, Northeast Forestry University, Harbin, China, 2Graduate School of Science, Kyoto University, Sakyo-ku, Kyoto, Japan, 3Department of Cell Biology, National Institute for Basic Biology, Okazaki, Japan

Higher plants accumulate storage proteins in seed as nitrogen as a nitrogen source for growth after germination nutrition for germination and growing. Seed storage proteins are synthesized on rough ER as precursors and then are transported to protein storage vacuoles for further processing into mature forms. We previously reported that *maigo2* mutant accumulated precursors of storage proteins and numerous novel structures-MAG2 Bodies, which are composed of storage protein precursors and ER chaperones within the ER lumen (1). MAG2 protein might be involved in the exit of storage protein precursors from the ER. In order to clarify mechanism underlying the MAG2-dependent transport, we studied interacting proteins with MAG2 and found three MAG2-interacting proteins (designated as MIPs). MIP proteins were associated with the ER membrane together with MAG2. *mip* mutants accumulated abnormally storage

protein precursors in their dry seeds. MAG2 body-like structures were also observed in *mip* seed cells. Our results suggest that MIP proteins cooperate with MAG2, playing a significant role in the transport of storage proteins in maturing seeds.

Li *et al*, Plant Cell 18: 3535–3547 (2006)

P013 A timing mechanism for stem cell maintenance and differentiation in Arabidopsis flower development

Bo Sun1,2, Yifeng Xu1, Kian-Hong Ng1, Toshiro Ito1,2,3

1Temasek Life Sciences Laboratory, 2National University of Singapore, 3PRESTO, Japan Science and Technology Agency

Strict control of stem cell activity ensures that plants of the same species have similarly sized flowers with a fixed number of floral organs. In flower development, the population of stem cells in floral meristem is terminated after the production of a fixed number of floral organ primordia. Precise repression of the *Arabidopsis thaliana* homeobox gene *WUSCHEL (WUS)* by the floral homeotic protein AGAMOUS (AG) plays a major part in this process. Both are transcription factors, but the transcriptional network that they control and the timing mechanisms by which they act remain to be understood. Here we show that KNUCKLES (KNU) mediates the repression of *WUS* in floral meristem determinacy control. AG directly induces the transcription of *KNU*, which encodes a C2H2-type zinc finger protein with a conserved transcriptional repression motif. In turn, KNU represses the *WUS* transcription to abolish stem cell activity. We further show that the timing of *KNU* induction is key in balancing proliferation and differentiation in flower development. Delayed *KNU* expression results in indeterminate meristem, whereas ectopic *KNU* expression prematurely terminates the floral meristem. Furthermore, we show that temporal regulation of *KNU* by AG depends on removal of the repressive histone modification H3K27 tri-methylation at the *KNU* locus. The histone demethylation precedes the transcriptional change of *KNU* that is AG-dependent. Together, these studies provide evidence for a mechanism by which the plant homeotic protein AG acts through temporal control of chromatin modification to control stem cell maintenance and differentiation.

P014 Expression control of the central growth regulator *BIG BROTHER* involves parallel function of independent transcriptional inputs

Holger Breuninger, Michael Lenhard

John Innes Centre Norwich

Organ size is genetically controlled. In plants, organ growth occurs in two phases: Firstly, primordium cells proliferate, before in a second phase they expand without further division. The switch between these two phases represents an important control point for the final size of organs. The Arabidopsis E3 ubiquitin ligase BIG BROTHER (BB) acts as a key repressor of cell proliferation. Organ size shows a tight inverse correlation with *BB* mRNA levels, such that mere two-fold changes in *BB* mRNA levels are sufficient to alter the final size of floral organs. Counter intuitively, the highest expression levels of *BB* are observed in proliferating tissue, suggesting that *BB* expression is controlled by growth stimulating pathways and acts in an incoherent feed-forward loop to prevent overproliferation.

We used promoter deletion analysis and phylogenetic footprinting to identify cis-regulatory elements within the *BB* promoter. Sequential deletions showed that at least two necessary but not sufficient cis-elements are located within 360 bp upstream of the *BB* transcriptional start site. Phylogenetic footprinting using eight species of Brassicaceae revealed at least 3 highly conserved motives within these 360 bp suggesting a conservation of partial redundancy in the upstream regulatory network. The identified elements are being used to isolate trans-acting factors important for *BB* expression and therefore organ growth control. Taken together, our results suggest a conserved functional interplay of independent regulators acting together to maintain stable levels of *BB* during cell proliferation.

P015 Auxin-independent regulation of *IAA12/BDL* expression during embryo development

Ive De Smet1,2, Steffen Lau1,2, Jasmin Ehrismann1, Ioannis Axiotis1,2, Marika Kientz1, Dolf Weijers1

1Center for Plant Molecular Biology (Developmental Genetics) / University of Tübingen, Auf der Morgenstelle3, D-72076 Tübingen, Germany, 2Max Planck Institute for Developmental Biology (Cell Biology), Spemannstrasse 35, D-72076 Tübingen, Germany

During plant development, the well-studied auxin-promoted degradation of AUX/IAA proteins that prevent ARF proteins from regulating auxin-responsive target genes is essential. However, very little is known about the transcriptional regulation of AUX/IAAs. To learn more about the transcriptional regulation of the *IAA12/BDL* gene, which is expressed in the apical daughter cell of the zygote and its progeny, we analyzed the *BDL* promoter. An initial *in silico* analysis followed by *in planta* validation revealed an evolutionarily conserved promoter fragment that is necessary and sufficient for *BDL* expression. Here, we present results on regulator Of BDL1 (ROB1), which functions as a direct repressor of *BDL* expression during embryo and root development. During embryo development, the spatial regulation of *BDL* expression appears to be crucial for the formation of cotyledons. Taken together, our results suggest that not only auxin-dependent degradation of AUX/IAAs controls auxin responses, but that also auxin-independent transcriptional regulation of AUX/IAAs is crucial for development.

P016 Regulation of floral patterning by flowering time genes

Chang Liu, Wanyan Xi, Lisha Shen, Hao Yu

National University of Singapore and Temasek Life Sciences Laboratory, Singapore

Flowers consisting primarily of four basic organ types are the most remarkable feature that characterizes the

angiosperms. Floral organ patterning in Arabidopsis requires activation of floral homeotic genes by the floral meristem identity gene, *LEAFY (LFY)*. Here we show that precise activation of class B and C homeotic gene expression in floral meristems is regulated by three flowering time genes, *SHORT VEGETATIVE PHASE (SVP), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1)* and *AGAMOUS-LIKE 24 (AGL24),* through direct control of a LFY co-regulator, *SEPALLATA3 (SEP3).* Loss of function of *SVP, SOC1* and *AGL24* derepresses *SEP3*, which in turn acts in concert with LFY to prematurely activate class B and C homeotic genes, thus resulting in striking floral defects such as loss of floral organs and generation of various chimeric floral structures. Orchestrated repression of *SEP3* by SVP, AGL24 and SOC1 is mediated by recruiting two interacting chromatin regulators, TERMINAL FLOWER 2/LIKE HETEROCHROMATIN PROTEIN 1 and SAP18, a member of SIN3 histone deacetylase complex. Our finding of coordinated regulation of *SEP3* by flowering time genes reveals a hitherto unknown genetic pathway that prevents premature differentiation of floral meristems and determines the appropriate timing of floral organ patterning.

P017 A link between ANGUSTIFOLIA3 and the adaxial/abaxial patterning of leaves through ribosome-related processes

Gorou Horiguchi1, Naoko Ishikawa2, Minoru Kubo3, José Manuel Pérez-Pérez4, María Rosa Ponce4, José Luis Micol4, Taku Demura3, Hiroo Fukuda 5, Hirokazu Tsukaya2,5

1Department of Life Science, College of Science, Rikkyo University, Toshima-ku, Tokyo, 171-8501, Japan, 2National Institute for Basic Biology, Okazaki, Aichi, 444-8585, Japan, 3RIKEN Plant Science Center, Yokohama, Kanagawa, 230-0045, Japan, 4Division de Genetica and Instituto de Bioingenieria, Universidad Miguel Hernandez, Campus de Elche, 03202 Elche, Alicante, Spain, 5Graduate School of Science, The University of Tokyo, Bunkyo-ku, Tokyo, 113-0033, Japan

Leaf development is dependent on appropriate adaxial/abaxial patterning and extensive growth. To investigate how these two processes are regulated, we characterized the role of *ANGUSTIFOLIA3 (AN3)* encoding a transcription coactivator. The *an3* mutations reduced cell proliferation without apparent defects in adaxial/abaxial patterning. Microarray analysis of *an3* demonstrated that many genes putatively involved in ribosome biosynthesis were down-regulated. Notably, the *an3* mutation enhanced a weak adaxial defect of the *asymmetric leaves2 (as2)* mutant. This was likely due to the reduced ribosome production since mutants defective in a ribosomal protein also enhanced adaxial defects in *as2*. Ribosome-related mutants often develop leaves with a pointed lamina. We have previously isolated a number of such mutants and many of them severely affected adaxial patterning when combined with *as2* while some of them did not have such an effect. These results suggest that *AN3* links growth and adaxial/abaxial patterning of leaves through ribosome biogenesis. In addition, ribosomal proteins might have unequal functions in adaxial/abaxial patterning of leaves.

P018 *AtNUFIP*: A key gene controlling the biogenesis of snoRNPs and scaRNPs directing methylation of rRNA and snRNA and its impact on plant development

Julie Rodor1, Edouard Jobet1, Jonathan Bizarro1, Christel Carles2, Manuel Echeverria1

1Université de Perpignan, UMR 5096 CNRS, Perpignan, France, 2IRD, UMR 5096, Montpellier, France

In eukaryotes small RNAs are essential for processing and modification of RNAs. *In vivo* they associate with several proteins and assemble into a functional RNP complex. In animals and yeast it has been shown that the assembly process is a key step and requires accessory proteins that transiently associate with the nascent RNP. Here we report on the identification of the Arabidopsis *AtNUFIP* gene encoding the first RNP assembly factor described in plants.

The *AtNUFIP* gene encodes a protein that has weak homology to the human NUFIP, which directs assembly of diverse RNPs in vertebrates. To investigate its role in Arabidopsis we isolated and characterised two *AtNUFIP* T-DNA insertional mutants. This showed that *AtNUFIP* specifically controls the biogenesis of two major RNPs: the C/D snoRNPs directing methylation of ribosomal RNAs in the nucleolus and the scaRNPs directing methylation of the spliceosomal snRNAs in the Cajal bodies. Most interesting this effect was strictly dependent on the genomic organisation of the small RNA components: *AtNUFIP* mutants are severely affected in the accumulation of polycistronic C/D snoRNAs or scaRNAs, but are not affected on either monocistronic C/D snoRNAs or intronic snoRNAs.

AtNUFIP mutants are viable but display severe developmental phenotypes. This raises the question whether this is due to a global decrease of methylation of rRNAs or snRNAs, or this is due to another associated activity. To answer these questions we are now producing transgenic lines that overexpress epitope tagged *AtNUFIP* to identify the protein partners and the RNA associated substrates.

P019 Epigenetic regulation of cartenoid composition and plant development by a chromatin modifying histone methyltransferase, SDG8?

Christopher Cazzonelli1, Barry Pogson2

1Center of Excellence in Plant Energy Biology, School of Biochemistry and Molecular Biology, Australian National University, Canberra, ACT, Australia, 2Center of Excellence in Plant Energy Biology, School of Biochemistry and Molecular Biology, Australian National University, Canberra, ACT, Australia

Carotenoid pigments are critical for the survival of plants and as a consequence carotenoid composition is finely tuned in response to the stage of development, tissue and external environmental stimuli. We have somewhat unexpectedly determined that the carotenoid isomerase (CRTISO) is a key regulatory step at the branch point in carotenoid biosynthesis and thereby alters the proportion of more than 4 different carotenoids. *CRTISO* is subject to regulation by a histone modifying enzyme, SET DOMAIN

GROUP8 (SDG8), that also regulates flowering time, seed set, germination, lateral root development and shoot branching. That is, we determined that permissive transcriptional regulation of *CRTISO* is enabled by histone 3 lysine 4 trimethylation of the *CRTISO* gene by cloning the carotenoid and chloroplast regulatory mutation (*ccr1*). Furthermore, regions within the promoter are required for SDG8 recruitment and function and the tissue specific expression of CRTISO is similar to that of SDG8. Mutants of SDG8 (*ccr1*) and CRTISO (*ccr2*) show an increase in shoot branching, which may be partly explained by limiting synthesis of the carotenoid-derived branching hormone, strigolactone. Microarray analysis of *ccr1* revealed a small number of gene expression changes, most downregulated (85), and primary targets of SDG8 are in the process of being determined to understand the role of SDG8 in controlling plant development. The essential roles that carotenoids and SDG8 play in root and shoot development have opened a new door towards understanding the regulation of carotenoid composition, potentially identifying novel carotenoid-derived signaling molecules and insight into epigenetic control of plant development. [Cazzonelli *et al*, 2009 in Plant Cell; 21(1):39-53 and Plant Signaling and Behavior; 4(4): 339-341].

P020 Polarised vascular cell divisions are controlled by the CLE41-PXY ligand-receptor pair

Peter Etchells, Simon Turner
The University of Manchester

Polarised cell divisions are essential for ordered eukaryotic development but little is known of the developmental cues by which this process is achieved. In plants, cambial cells generate vascular tissue in a highly ordered process of coordinated polar cell divisions along their long axis. These polar divisions result in files of cells which are displaced from the meristem and subsequently differentiate into xylem towards the centre of the stem and phloem towards the periphery. The receptor kinase PHLOEM INTERCALATED WITH XYLEM (PXY) was identified as being essential for ordered, coordinated cell divisions in the Arabidopsis procambium. In the absence of PXY, the signal that sets the division plane is disrupted resulting in the loss of clearly visible cell files. PXY was the first example of a receptor kinase being essential for determining the cell division plane and several receptor kinases have subsequently been identified which are required for other highly ordered cell divisions. ACR4, PAN1 and SUB receptors are involved in specifying cell divisions in lateral root initiation, stomatal development, and apical meristems, respectively. Ligands for these receptors are unknown, and as such, their role in these processes is yet to be determined. In contrast, it has been recently been shown that a member of the CLE family, CLE41, is the ligand PXY. High levels of CLE41 result in an increase in vascular cell number, suggesting that the PXY-CLE41 interaction is part of a pathway that influences the balance between meristematic and differentiated cells. However, a more dramatic phenotype occurs when positional information conferred by the ligand is disrupted. In these plants, complete loss of organisation of the vascular tissue within the hypocotyl results in interspersal of the normally well-separated xylem and phloem and a loss of orientation of the xylem along the apical basal axis. This data demonstrates that localised expression of *CLE* genes in the phloem adjacent to those expressing PXY provides positional information that is essential for orientating the plane of cell division during normal vascular organisation.

P021 In Arabidopsis, a novel binding site for AP2 is important for AG regulation

Thanh Theresa Dinh, Xuemei Chen
University of California, Riverside, Riverside, Ca, USA

One of the first steps of cell fate patterning is the regulation of gene expression, specifically by way of transcription factors. In Arabidopsis, there are four classes of homeotic genes, A, B, C, and E, which control proper floral organ identity. Genetic studies reveal that these four classes function in a combinatorial and cadastral manner to confer proper floral identity. Specifically, A-class gene, *APETALA2 (AP2)* functions to promote sepal and petal identity in whorls 1 and 2, and to restrict the expression of the C-class gene, *AGAMOUS (AG)*, from whorls 1 and 2; however, it is unknown how AP2 performs these functions. Furthermore, unlike the other floral genes which contain MADS domains, AP2 itself has two DNA binding domains termed the AP2 domains, AP2R1 and AP2R2. Interesting, MADS-domain containing proteins have been highly characterized and its targets have been identified, yet for AP2, its target binding sequence is not yet known nor have targets been identified. Here, using biochemical *in vitro* assays, we show that AP2 binds a non-canonical target sequence. Furthermore, utilizing GUS reporter assays with truncations of the AG second intron, we found that this sequence is also important for the restriction of *AG* expression, *in vivo.*

P022 Establishing regulatory models for anther endothecium development and the regulation of dehiscence

Caiyun Yang, Jie Song, Zoe A Wilson
University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

The endothecium plays a critical role during late anther development and pollen release. Post-meiosis this maternal cell layer undergoes deposition of bands of secondary thickening. Immediately prior to dehiscence, differential swelling followed by desiccation-induced shrinkage of thickened and unthickened regions of the endothecial cell wall result in an outward bending force leading to retraction of the anther wall and pollen release. The secondary thickening is vital for this to occur, as shown by the indehiscent *ms35* mutant, which fails to produce secondary thickening in the endothecium.

Secondary cell walls are composed of cellulose, hemicellulose and lignin. Advances have been made towards understanding the pathways of cellulose, lignin and xylan biosynthesis, however identifying the regulatory factors controlling them, particularly tissue specificity, is still in its

infancy. We have shown that MYB26 is a regulator of secondary thickening that can ectopically induce thickening in other tissues (Yang *et al*, 2007). This indicates that the down-stream mechanisms for secondary thickening are conserved, but that the trigger for such events is due to specific regulators, for example MYB26, which determines temporal and tissue specificity in the anther.

We have used transcriptomics to identify components of the MYB26 regulatory network and systems biology approaches have been applied to generate preliminary network models. Overexpression of downstream MYB26 targets in the *myb26* mutant has shown that MYB26 functions in defining endothecium development, as well as in the regulation of secondary thickening. The role that MYB26 plays in endothecial development and in the regulation of genes associated in secondary thickening will be presented.

Yang *et al* (2007). The Arabidopsis *MYB26/MS35* gene regulates secondary thickening in the endothecium and is essential for anther dehiscence. The Plant Cell 19, 534-548.

P023 ChIP-Seq and inducible gene expression reveal direct targets of the flowering pathway integrator FD

Levi Yant, Anusha Srikanth, Felix Ott, Christa Lanz, Frank Küttner, Markus Schmid

Max Planck Institute for Developmental Biology, Tubingen, Germany

Rooted in place, flowering plants must respond correctly to the environment. Distinct pathways relate information to modulate the expression of floral integrator genes, allowing expression only when the time is right for reproduction. FD is one such floral integrator that complexes with FT, a mobile signal which induces flowering. How the FD/FT module functions is not well understood. We are therefore investigating FD targets with genome-wide assays.

ChIP-Seq couples chromatin immunoprecipitation(ChIP) to massively parallel sequencing to create *in vivo* genome-wide maps of DNA-protein interactions. Rapidly eclipsing ChIP-chip, which employs tiling arrays, ChIP-Seq promises higher positional resolution, fewer false positives, and decreased expense.

We generated ChIP-Seq and -chip data for FD tagged with GFP. Between 4 and 9 million high quality reads from an Illumina 1G genome analyzer were mapped from each run and analyzed for enrichment across the genome. High confidence (FDR Q<10^-20) binding to MADS-gene loci *SEPALLATA3(SEP3*), *APETALA1 (AP1*), and *FRUITFULL* was detected, along with binding to other flowering-related loci. ChIP-chip data were largely consistent with ChIP-Seq data. *de novo* Gibbs sampling showed enrichment for a G-box motif in bound regions. Binding to this motif in the *SEP3* promoter was confirmed by EMSA and mutation of two core nucleotides abrogated binding.

To distinguish between direct, proximal, and indirect targets, we performed gene expression analyses with an inducible system. Consistent with ChIP-Seq data, *SEP3* and *AP1* were indicated as direct targets, while other flowering related genes were induced later. These results shed light on how a pathway integrator orchestrates a major developmental transition in plants.

P024 Control of embryo development by the CUL4-DDB1 complex

Eva Dumbliauskas, Jean Molinier, Pascal Genschik

CNRS-IBMP (Institut de Biologie Moléculaire des Plantes), Strasbourg, FRANCE

Regulation of protein stability through the ubiquitin proteasome system (UPS) is now considered as a major mechanism underlying many cellular and organismal processes, such as cell division, DNA repair and epigenetic phenomenon, developmental pathways, important parts of immune defence and in plants, light and phytohormone signal transduction. Degradation "via" the UPS is a two-step process: the protein is first tagged by covalent attachment of ubiquitin and subsequently degraded by a multicatalytic protease complex called the 26S proteasome. Conjugation of ubiquitin to the protein is achieved through an enzymatic cascade involving the sequential action of three enzymes: E1, E2 and E3. The E3 enzymes play the most important role by bringing the specificity to the system. Several hundred different E3s have been identified; among them Cullin (CUL)-dependent ubiquitin ligases are the most intensively studied. The CUL4-DDB1 ligases belong to this class of enzymes and participate in the maintenance of genome integrity in different organisms. Contrary to human, *Arabidopsis thaliana* contains only one *CUL4* gene, but two *DDB1* genes, called *DDB1A* and *DDB1B*. *CUL4*-containing protein complexes often associate with WD40 domain proteins that act as substrates and/or as adaptors for this E3 class of enzymes. MSI1 is a WD40 domain protein, which is involved in parental genome imprinting, among other functions, and which contains the recently identified DxR motifs necessary for DDB1 interaction.

We will present here the first characterization of Arabidopsis *cul4* and *ddb1* loss of function mutants and discuss the interaction between CUL4-DDB1 and MSI1.

P025 Ribosome heterogeneity in the plant cell - what is its function?

Peta Bonham-Smith

University of Saskatchewan

Why do plants have a level of heterogeneity in ribosome composition that is not seen in animal, fungal and bacterial ribosomes? Unlike animals, fungi and bacteria, each protein (RP) of the plant cytosolic ribosome is encoded by a multi-gene family, resulting in a number of isoforms of each RP. However, other than the acidic P-RPs (P1, 2a,2b,3), only one copy of each RP is found in each ribosome. Focusing on one small subunit (SSU: RPS15a – 6 genes [1 pseudogene] = 4 isoforms) and one large subunit (LSU: RPL23a – 2 genes = 2 isoforms) RP in Arabidopsis we have started to identify some of the differences between RP isoforms.

RPS15a (bacterial orthologue RPS8) is a primary binder of SSU 18S rRNA. Transient expression of fluorescent-tagged RPS15a isoforms has demonstrated differential

localisation of RPS15aA/F and –D (Type I) to the nucleolus (site of cytosolic ribosome subunit assembly) and RPS15aB and –E (Type II) to mitochondria. Using ElectroMobility Shift Assays (EMSAs) we have further demonstrated differential binding of Type I and Type II RPS15as to cytosolic and mitochondrial SSU 18S rRNAs. Type I RPS15as will only bind cytosolic 18S rRNA while Type II RPS15as will bind both cytosolic and mitochondrial SSU rRNAs. RPL23a (bacterial orthologue RPL23) is located at the exit of the peptide tunnel of the ribosome and thought to interact with the ER translocon. We have shown, through RNAi, that RPL23aA is absolutely required for plant viability. As yet we have not identified a role for RPL23aB. We have proposed that L23a is required for ribosome biogenesis through a role in rRNA processing within the nucleolus. Transient expression of fluorescent-tagged RPL23aA and -B demonstrates a preferred nucleolar localization for RPL23aA (contains a putative nucleolin binding site) and nucleolar exclusion of RPL23aB. Site Directed Mutagenesis (SDM) of the nucleolin binding site in RPL23aA results in it being excluded from the nucleolus. We are currently assessing RPL23aA and –B interactions directly with nucleolin.

Ribosome composition may regulate translation specificity, therefore, understanding RP isoform composition of the ribosome could lead to 'customised' ribosome composition directing optimised ribosome function.

P026 The *KAONASHI4* gene encoding a putative β1,3-galactosyltransferase is required for the thickening of the pollen exine structure in *Arabidopsis thaliana*

Toshiya Suzuki, Kenzo Nakamura, Sumie Ishiguro

Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan

The exine composing an outer wall of pollen grains is believed to be important for successful pollination because its architecture shows high diversity among higher plants. However, little is known about the mechanism of exine formation. To identify the novel genes involved in it, we performed the screening of mutagenized Arabidopsis with SEM, and identified many exine defective mutants. Among them, we focused on the *kaonashi4* (*kns4*) showing thin exine layer but normal reticular pattern. Map-based cloning revealed that the *KNS4* gene encodes a putative β1,3-galactosyltransferase (GalT) involved in glycan biosynthesis. By TEM analysis, we found that the quantity of primexine, the scaffold of exine formation, was reduced at the tetrad stage in *kns4*. RT-PCR and promoter-reporter analyses indicated that the *KNS4* is specifically expressed in the tapetal cells around tetrad stage. Arabidopsis has 20 putative β1,3-GalT isoforms and they are divided into 4 subfamilies. The *KNS4* belongs to a subfamily including 8 GalTs that have low homology with animal ones, and its orthologues widely spread among land plants. In animals, β1,3-GalTs are only involved in complex N-glycan biosynthesis. In contrast to the large number of β1,3-GalT genes in Arabidopsis genome, most of the enzymes of this pathway are encoded by a single or small number of genes. We found that the knockout mutants for these enzymes never showed exine abnormalities. These results strongly suggested that *KNS4* does not work in the pathway of complex N-glycan biosynthesis. Our hypothesis is that *KNS4* functions in tapetal cells at tetrad stage and is involved in biosynthesis and/or secretion of the components of primexine.

P027 Coordination of dispersed stem cell activity in Arabidopsis

Derek White

AgResearch, Grasslands Research Centre, New Zealand

Vascular plants have the capacity to vary their patterns of secondary growth in response to different environmental conditions. While it is presumed that this plasticity in growth is coordinated in some way, there is very limited information about the genetic mechanisms that are involved. Much of the secondary growth in higher plants originates from populations of dispersed stem cells (DSC), most notably the procambium and cambium that produces the vascular tissues and the shoot epidermal meristemoid mother cells (MMC) that initiate the stomatal lineage. The activity of procambium/cambium is a major determinant of vascular growth and the activity of the MMC's during epidermal development determines stomatal density. Previously there has been no indication that the activities of these different stem cell populations are coordinately regulated.

In Arabidopsis deletion of the *PEAPOD (PPD*) locus results in enlarged dome shaped leaves and wide siliques (1). This excess lamina growth in *ppd* loss-of-function mutant plants is due to extended proliferation of MMCs. The *ppd* mutant also has thickened roots, hypocotyls and stems, all due to an increase in procambium activity producing increased vascular growth throughout the plant. The *PPD* locus is made up of two orthologs, *PPD1* and *PPD2*, with either gene able to complement the entire phenotype of the mutant. Over expression of *PPDpro-PPD* results in both reduced procambium and MMC activities and a coordinated reduction in both vascular and stomatal densities. The *PPD* genes therefore act to regulate and coordinate vascular and stomata density by limiting the activity of procambium/cambium and MMC stem cells. *PPD* homologs appear to be restricted to plants that have a vascular cambium.

White, D.W.R. (2006) PNAS 103: 13238-13243.

P028 Establishment of the winter-annual growth habit by *FRIGIDA*-mediated histone methylation in Arabidopsis

Danhua Jiang1,2, Xiaofeng Gu1, Yuehui He1,2

1National University of Singapore, Singapore, 2Temasek Life Sciences Laboratory, Singapore

The transition to flowering is a major developmental switch in the plant life cycle that is properly timed to achieve reproductive success. In *Arabidopsis thaliana,* naturally-occurring flowering-time variation exists among wild accessions. The winter-annual (late-flowering without vernalization) versus rapid-cycling (early flowering) growth habit is typically determined by allelic variation at *FRIGIDA (FRI)* and *FLOWERING LOCUS C (FLC)*. *FRI*

upregulates the expression of *FLC*, a central floral repressor, to levels that inhibit flowering, resulting in the winter-annual habit. To date, how *FRI* upregulates *FLC* expression is not known. Here we show that *FRI* specifically promotes histone H3 lysine-4 trimethylation (H3K4me3, an active chromatin mark) at the *FLC* locus to upregulate its expression. We have identified a WD-domain protein that is required for *FRI*-mediated *FLC* upregulation. We found that *FRI* specifically mediates the enrichment of this protein at the *FLC* locus, leading to elevated H3K4me3. In addition, we found that in the presence of a functional FRI, the activity of an *FLC* repressor known as *FLD*, which mediates H3K4 demethylation in *FLC* chromatin, is compromised at the *FLC* locus. Our findings suggest that *FRI* mediates the enrichment of a WD-domain protein at the *FLC* locus, leading to elevated H3K4me3 and thus *FLC* upregulation to establish the winter-annual growth habit.

P029 Morphology and molecular regulation of programmed cell death in xylem elements

Charleen Courtois1, Edouard Pesquet1, Sunil Kumar Singh1, Benjamin Bollhöner1, Minako Kaneda2, Lacey Samuels2, Hannele Tuominen1

1Umea Plant Science Centre, Department of Plant Physiology, Umea University, 90187 Umea, Sweden, 2Department of Botany, University of British Columbia, Vancouver, BC V6T 1Z4, Canada

The last stage of xylem development is programmed death of the cells, followed by complete autolysis of the cell contents. Two cell types predominate in the xylem; the vessel elements and the fibers. We have described a unique type of cell death program in xylem fibers of hybrid aspen *(Populus tremula x tremuloides)* stems including gradual degradative processes in both the nucleus and cytoplasm leading into loss of the cytoplasmic contents well before the loss of vacuolar integrity, which is considered to be the moment of death. This type of cell death differs significantly from that seen in the xylem vessels. High-resolution microarray analysis in the vascular tissues of Populus stem, combined with *in silico* analysis of publicly available data repositories, points to the involvement of several previously uncharacterized transcription factors, ethylene, sphingolipid and light signaling as well as autophagy in the control of fiber cell death. We have characterised the function of several genes, including a bifunctional nuclease and a metacaspase, in xylem cell death using a reverse genetic approach in Arabidopsis. Some of these results will be described here.

P030 CKH1/EER4/AtTAF12b and CKH2/PKL may function together to regulate cytokinin responses of calli in Arabidopsis

Kaori Furuta1, Minoru Kubo2, Taku Demura3, Tatsuo Kakimoto1

1Osaka University, Japan, 2National Institute for Basic Biology, Japan, 3RIKEN, Japan

Cytokinins promote cell division and chloroplast development in tissue culture. We have previously isolated two Arabidopsis mutants, ckh1 *(cytokinin-hypersensitive1)* and *ckh2*, which exhibit cytokinin-hypersensitivity for callus growth. *CKH1* encodes EER4 /TAF12b (TBP ASSOCIATED FACTOR 12b). *CKH2* encodes PKL, which resembles CHD3 class SWI/SNF2 family chromatin remodeling factors of yeast and animals. A microarray experiment revealed that many genes involved in photosynthesis were more sensitively induced by cytokinins in these mutant calli than in WT calli. The *ckh1ckh2* double mutant produced green calli with only auxin without cytokinin, and cytokinins did not affect callus growth. This synergistic effect of two mutations suggests that CKH1 and CKH2 may function in the same pathway. A yeast two hybrid experiment showed protein interaction between CKH1 and CKH2, suggesting that CKH1 and CKH2 may act together, perhaps on genes that can be regulated by cytokinins.

P031 Paternal regulation of maternal gene expression may provide adaptive developmental strategies in Arabidopsis

Maria Cartagena, Jonathan FitzGerald

Rhodes College, Memphis, TN USA

Variations in seed size between three Arabidopsis ecotypes may be regulated by parental genomic imprinting (PGI) or a similar epigenetic mechanism. PGI silences either the paternal or maternal allele of a gene after fertilization and is regulated in part by the activity of the *FIS* Polycomb group (PcG) histone methyltransferase. Seed sizes in crosses between Landsberg *erecta* (Ler), Columbia (Col) and C24 strains correlated more strongly with the choice of parent than the resulting genotype of the seed itself. To determine correlation to PcG targets, we examined expression of the Arabidopsis formin *AtFH5* using a C24 GFP enhancer trap inserted in the endogenous *AtFH5* promoter. *AtFH5* is imprinted by FIS PcG in the seed endosperm resulting in maternal expression after fertilization. Paternal contribution from Ler or Col ecotypes reduces maternal GFP expression. This effect is increased by 25% of segregating F1 Col x Ler pollen, suggesting independent repressive pathways. When Col x Ler recombinant inbred (RI) lines where crossed as fathers to the AtFH5 reporter, we identified quantitative trait loci (QTL) for both seed size variation and influence on maternal GFP expression. QTL for paternally derived traits were mostly distinct from traits in selfed RI lines, however one QTL was shared between seed size and the paternal regulation of maternal GFP levels. We hypothesized that these effects in hybrid seed development might represent a miscommunication between maternal and paternal 'cross-talk' in the selected ecotypes. Accordingly, the onset of flowering was delayed for as long as 2 months in hybrid F1s compared to parental strains. Flowing time is epigenetically regulated and involves vegetative PcG activity. Features of these non-flowering plants included a leaf morphology at high temperatures that resembles loss of vegetative PcG complex member *CURLY LEAF.* We propose that in addition to evolution through DNA mutation, epigenetic selection of PcG target genes represents a short term, and possibly reversible, adaptive developmental strategy employed by populations in distinct environments.

P032 Global identification of targets of the MADS-domain protein AGL15

Yumei Zheng, Sharyn Perry

Univ. of Kentucky, Dept. of Plant and Soil Sciences, Lexington, KY, USA

AGL15 is a member of the MADS-domain family of transcriptional regulators that accumulates to its highest amounts during embryogenesis. Ectopic expression of *AGL15* promotes somatic embryogenesis, whereas loss-of-function shows significant reduction in production of somatic embryos. AGL15 promotes embryogenesis by controlling downstream gene expression but little is known about directly regulated targets. Chromatin immunoprecipitation (ChIP) has been used to identify DNA fragments with which AGL15 is associated *in vivo* and a low-throughput approach to identify these fragments has revealed a role for AGL15 in GA catabolism that is relevant to embryogenesis. However, to understand more globally the gene networks in which AGL15 is involved, higher throughput methods to identify direct and indirect targets are needed. ChIP-on-chip using the Affymetrix Arabidopsis tiling arrays was performed to map *in vivo* binding sites for AGL15 and approximately 2000 sites represented in 3 biological replicates of the experiment were annotated to nearby genes. These results were combined with high-throughput measurement of gene expression in response to *AGL15* accumulation using the Affymetrix ATH1 arrays to discriminate responsive direct targets from those further downstream in the network. A summary of numbers and types of genes controlled by AGL15 will be presented. Other transcriptional regulators are over-represented in the directly expressed set of targets. Interestingly, *LEC2, FUS3*, and *ABI3*, that encode B3 domain transcription factors that are key regulators of embryogenesis were identified and verified as directly expressed target genes of AGL15. Additionally, genes identified as targets of the B3 domain proteins are also targets of AGL15 and we have found that IAA30 is involved in promotion of somatic embryo development. Results from global analysis of targets have indicated a possible role for hormone interactions in promotion of somatic embryo development. Our and others data suggest that there will be high amounts of cross-regulation in gene regulatory networks underpinning embryogenesis.

P033 Chloroplast biogenesis and retrograde signalling in seedlings

Veronica Albrecht1, Klaus Apel2, Murray Badger1, Barry Pogson1

1Australian National University Canberra, Australia, 2ETH Zurich, Switzerland

The formation of functional chloroplasts during seedling development is critical for normal development and ultimately yield of the plant. The development and activity of chloroplasts has been shown to differ in cotyledons that initially serve as a storage organ in contrast to true leaves, whose primary function is photosynthesis by the identification of multiple nuclear-encoded *snowy cotyledon* (*sco*) and *white cotelydon (wco)* mutants. Analysis of the *sco* mutants led to the identification of chloroplast-targeted proteins involved in protein translation as *sco1* is a mutation in the plastid elongation factor G and protein folding as *sco2* is mutated in a protein disulphide isomerase. Surprisingly, *sco3* implicates a third organelle is involved in the regulation of chloroplast biogenesis since *SCO3* encodes a peroxisome-localized protein of, as yet, unknown function. Evidence that *SCO3's* primary role is not in peroxisome metabolic activity but cellular signalling will be discussed. The consequences of the *sco* mutations include alterations in plastid size and ultrastructure, however, each has unique phenotypes and affects different stages of plastid differentiation whether from proplastid to chloroplast or proplastid to etioplast to chloroplast. Thus, despite the *sco* mutants showing a similar primary phenotype of pale cotyledons but green true leaves, the different mutations affect diverse aspects in chloroplast biogenesis in cotelydons and leaves.

P034 TCTP, a putative upstream component of the TOR signalling pathway, controls tip growth and root development in Arabidopsis

Oliver Berkowitz1,2, Ricarda Jost1,3, Stephan Pollmann4, Josette Masle1

1The Australian National University, Canberra ACT 0200, Australia, 2Murdoch University, Murdoch WA 6150, Perth, Australia (present address), 3The University of Western Australia, Crawley WA 6009, Perth, Australia (present address), 4Ruhr-University Bochum, 44801 Bochum, Germany

The TOR (target of rapamycin) signalling pathway is the major regulator of cellular growth in animals and fungi. The TOR Ser/Thr kinase as the central component integrates upstream inputs (e.g. nutrient and energy status) to regulate downstream processes (e.g. ribosome biogenesis, translation efficiency), thus tuning the increase in cytoplasm mass to internal and environmental stimuli. Alterations in TOR activity lead to changes in cell growth and subsequently organ size. A similar picture also emerges for plants, although only few components of the plant TOR network have been identified so far. Here we present evidence that the translationally controlled tumor protein (TCTP) from Arabidopsis, as its counterpart in non-plant systems, acts as an upstream activator of TOR. TCTP expression is ubiquitous throughout tissues but is increased in actively dividing and proliferating cells. Knock-out of the *TCTP* gene by T-DNA insertion leads to a male gametophytic defect with impaired pollen tube growth and a reduction in competitiveness of mutant against wild-type pollen. Down-regulation of *TCTP* expression by RNAi results in a reduction in cell sizes, leading to retarded vegetative growth, and also alterations in root development, root hair morphology and lateral root formation. Analyses of GFP reporter lines corroborate these findings. Furthermore plants silenced in *TCTP* expression show an increased auxin resistance with elevated endogenous auxin levels. These results reveal the importance of TCTP for plant development and are consistent with its proposed function in the TOR signalling network.

The financial support of GRDC for this work (grant ANU08 to JM) is gratefully acknowledged.

P035 The MAR binding protein AHL16 controls flowering time by regulating chromosome structure of the *FLC* locus

Yifeng Xu1, Toshiro Ito1,2

1Temasek Life Sciences Laboratory, 2PRESTO, Japan Science and Technology Agency

Flowering Locus C (FLC) is a flowering repressor and is the integrator of multiple flowering pathways in Arabidopsis. The transcriptional regulation of *FLC* is well controlled at several levels, including chromatin regulation. Here, we introduce AHL16, an AT-hook region binding nuclear localized protein, into the network controlling the *FLC* transcriptional level. Knocked down of AHL16 causes extremely delayed flowering, as well as altered phyllotaxis and apical dominance. Expression analysis indicates that *FLC* is dramatically increased in the AHL16 knocked-down transgenic plants compared with the wild-type plants. The AHL proteins were supposed to bind to matrix attachment region (MAR) by AT-hook motif and be located on the chromatin surface during mitosis. Five putative MAR elements were found in the genomic regions of *FLC* and its neighboring genes. MAR analysis indicates that the structure of *FLC* genomic region was changed in the AHL16 knocked-down transgenic plants, suggesting AHL16 regulates *FLC* expression level by modifying the chromosome structure.

P036 Arabidopsis SHEPHERD, an Hsp90-like molecular chaperone resident in the endoplasmic reticulum, buffers against a harmful genetic mutation in *CLAVATA2*

Tomoko Niwa1, Kumiko Oba1,2, Kenzo Nakamura1, Sumie Ishiguro1

1Graduate School of Bioagricultural Sciences, Nagoya University, Nagoya, Japan, 2Current address: ERATO, Japan Science and Technology Agency, Okazaki, Japan

The Arabidopsis *SHEPHERD (SHD)* gene encodes the putative ortholog of GRP94, an Hsp90-like molecular chaperone resident in the endoplasmic reticulum. The *shd* mutant shows pleiotropic phenotypes including clavata (*clv*)-like phenotypes, in which shoot apical and floral meristems are enlarged and floral organ numbers are increased. Therefore we presumed that the *SHD* is required for functional maturation of one or more components in CLV signaling pathway. We found that this *clv*-like phenotype of *shd* mutation appears only in some genetic background including Wassilewskija-2 (Ws), and never in the background of many accessions including Columbia-0 (Col) and Landsberg erecta. This meant that there is an enhancer mutation that makes *shd* show *clv*-like phenotype. Genetic analysis revealed that the enhancer mutation is located in the *CLV2* allele in Ws (*CLV2-Ws*). Exogenous CLV2-Col complemented the *clv*-like phenotype of *shd* mutants in Ws background. We concluded that the CLV2-Ws is dependent for the chaperone activity of SHD. There are seven amino acid substitutions between CLV2-Ws and CLV2-Col, and we identified one of them is responsible for the SHD dependency. On the basis of these results, we hypothesize that SHD buffers against harmful mutations of *CLV2*, which may be accumulated as genetic variations.

P037 Interaction between the ubiquitination and the miRNA pathways in the regulation of flower development

Zinnia Haydee Gonzalez-Carranza1, Xuebin Zhang2, Janny L Peters3, Jeremy A Roberts1

1The University of Nottingham, England, UK, 2Rothamsted Research, UK, 3Radboud University Nijmegen,

The Netherlands

HAWAIIAN SKIRT (HWS) is an ubiquitin protein ligase (SCF~*HWS*) that plays a role in regulating flower development in Arabidopsis (Gonzalez-Carranza, *et al*, 2007). Although the mutant is pleiotropic, the most conspicuous feature is the fusion of its sepals, which precludes floral organ shedding. This phenotypic characteristic resembles that observed in the double mutant of *cuc1/cuc2* (Aida, *et al*; 1997) and the ectopically expressing lines of the microRNA 164b (Laufs, *et al*, 2004; Mallory, *et al*; 2004).

In an attempt to identify potential substrates for HWS and dissect downstream events, several strategies have been pursued. These have included phenotypic analyses after crossing *hws* with other mutants that display floral phenotypes, Y2H analyses using HWS as a bait, and the isolation and characterization of suppressors after generating an EMS mutagenized *hws-1* population.

An initial screening of the EMS mutagenized *hws-1* population has identified several suppressors lines. One of these (named *prb-1*) has proved to be a dominant mutation. Not only does *prb-1* rescue the sepal fusion phenotype in *hws-1* plants it also exhibits an increase in the number of sepals, petals, and anther filaments. Intriguingly, this *prb-1* characteristic of elevated numbers of floral organs is less marked in a wild type background. The mutant *prb-1* has been mapped and the nature of the mutation identified as a single nucleotide substitution in a NAC gene. This mutation is sited in the middle of a microRNA binding domain and provides evidence that HWS may influence flower development via the gene silencing machinery. These observations will be discussed.

Aida, *et al*, (1997). The Plant Cell. 9: 841-857.
Gonzalez-Carranza, *et al,* (2007). Plant Physiology. 144:1370-1382.
Laufs, *et al*, (2004). Development, 131: 4311-4322.
Mallory, *et al*, (2004). Current Biology. 14: 1035-1046.

P038 Ribosomal proteins regulate Arabidopsis leaf dorsoventral polarity

Pascale Rossignol1, Peter Etchells2, Mary Byrne1

1John Innes Centre, Norwich, UK, 2Manchester University, UK

In plants, leaves are a major site of carbon fixation. An efficient way of maximising light capture and carbon fixation is for the leaves to develop as plane structures. In our lab, we are focusing on the characterisation of components involved in the establishment and maintenance of leaf dorsoventral polarity using the plant model *Arabidopsis thaliana*. One key player in leaf patterning is the MYB-transcription factor *ASYMMETRIC LEAVES 1 (AS1)* (Byrne *et al* 2000). A screen for modifiers of *as1* revealed

mutants displaying ectopic leaves on the adaxial side of the main leaf ("piggyback" phenotype): a hallmark of defects in leaf dorsoventral polarity. Mutations in ribosomal protein genes are responsible for this phenotype (Pinon, Etchells *et al* 2008). This suggests that ribosome function is important for proper leaf development and more interestingly also raises the question as to whether the particular defects seen in these mutants are a consequence of reduction in global translation and/or a mis-regulation of specific target genes.

To answer these questions, we are using a combination of biochemical and genetic approaches. So far, this work has led us to two main hypotheses, which could explain that, in addition to housekeeping functions, ribosomal protein genes may also able to specifically regulate the expression of target genes via functional specialisation of ribosomal paralogues and/or interaction with non ribosomal proteins.

Byrne, M. E., *et al* (2000). Asymmetric leaves1 mediates leaf patterning and stem cell function in Arabidopsis. Nature 408(6815): 967-71.
Pinon, V., J. P. Etchells, *et al* (2008). Three PIGGYBACK genes that specifically influence leaf patterning encode ribosomal proteins. Development 135: 1315-1324.

P039 Functional analysis of CYP98A8 and CYP98A9 in Arabidopsis

Michiyo Matsuno, Vincent Compagnon, Nawroz Abdul Razzak, Daniéle Werck
IBMP-CNRS, Strasbourg, France

Cytochrome P450 (P450s) is one of the largest enzyme families in higher plants. P450s are involved in the biosynthesis of various secondary metabolites related to defense responses or developmental events. Enzymes from CYP98 family are known to play a key role in lignin biosynthesis. In Arabidopsis, CYP98A3 (At2g40890) possesses the major function, located in xylem part of the stem and catalyzes 3'-hydroxylation of p-coumaroyl shikimate or p-coumaroyl quinate to form monomers precursor of lignin. However its orthologues CYP98A8 (At1g74540) and CYP98A9 (At1g74550) have distinct location. Opposingly to other members of CYP98 family they do not metabolize p-coumaroyl shikimate or p-coumaroyl quinate and their functions still remain to be established.

By promoter-GUS analysis, we showed that CYP98A8 and CYP98A9 are expressed in early developmental stage of young flower, especially in tapetosome and CYP98A9 is expressed in root tip. In the comparison of the metabolic profiling of various transgenic plants, we showed that a metabolite is missing and a new metabolite appears in CYP98A8KO. By UPLC-MS/MS we identified a polyamide as a substrate of CYP98A8. We also found a new metabolite in *CYP98A8/CYP98A9* RNAi and identified another polyamide as a substrate of CYP98A9. We are now exploring their biological functions in the pollen and the roots.

P040 A systems approach reveals new roles for SHORTROOT and SCARECROW in plant development and physiology

Hongchang Cui, Mikhail Kovtun, David Orlando, Philip Benfey
Duke University, Durham, NC, USA

SHORTROOT (SHR) and SCARECROW (SCR) are key regulators of root growth and development in *Arabidopsis thaliana*. Made in the stele, the SHR protein moves into an adjacent cell layer where it activates *SCR* transcription. SHR and SCR together in turn define a single layer of endodermis, and maintain the stem cell niche. SHR and SCR are also essential for the formation of the starch sheath in the stem, which contains amyloplasts that play an important role in gravitropism. Although *shr* and *scr* mutants are defective in amyloplast development and gravitropic responses in the shoot, it is not clear whether this is due to the loss of the starch sheath cell layer or through some other mechanisms. SCR is expressed in the shoot apical meristem (SAM) and in aerial organs, but its function in these tissues is not well characterized.

To dissect the SHR/SCR developmental pathway, we have determined the genomewide locations of SHR direct targets using a ChIP-chip method that we independently developed. All known SHR targets were identified, most of which were among the 25 top-ranked genes. GFP transcriptional fusion studies showed that some top-ranked SHR targets of unknown function are expressed in the endodermis, further validating our approach. Interestingly, these genes are not expressed throughout the endodermis, but are expressed at different stages. Intriguingly, some SHR targets appear to be preferentially expressed in the pericycle and xylem, suggesting that SHR might have a broad role in cell fate specification in the root. Among the top-ranked SHR targets there are also several stress-associated genes, and we found that both the *shr* and *scr* mutants have altered stress responses. Finally, we will present evidence that suggests an important role for SCR in integrating environmental stimuli with plastid development.

P041 The role of auxin in Arabidopsis late stamen development

Valentina Cecchetti1, Giuseppina Falasca1, Maria Maddalena Altamura1, Karin Ljung2, Paolo Costantino1, Maura Cardarelli3
1Université La Sapienza, Rome, Italy, 2Umea University, Umea, Sweden, 3IBPM-CNR, Université La Sapienza, Rome, Italy

Based on an extensive genetic and molecular analysis in Arabidopsis, we have proposed a model on the role of auxin in late stamen developmental processes: anther dehiscence, pollen maturation and filament elongation. Expression of auxin-sensitive reporter constructs indicates that auxin effects begins in anthers between the end of meiosis and the bilocular stage. *In situ* hybridizations of the auxin biosynthetic genes *YUC2* and *YUC6* indicate that auxin is synthesized in anthers. In agreement with the timing of auxin effects, the *TIR1, AFB1, AFB2*, and *AFB3* auxin receptor-encoding genes are transcribed in anthers only during late stages of development starting

at the end of meiosis. In *tir1 afb* quadruple mutants, anther dehiscence and pollen maturation occur earlier than in the wild type, causing the release of mature pollen grains before the completion of filament elongation. We also assessed the contribution of auxin transport to late stamen developmental processes by analysing the *mdr1 pgp1* mutants affected in auxin transport, and show that while auxin synthesized in anthers plays a major role in coordinating anther dehiscence and pollen maturation, auxin transport contributes to the independent regulation of preanthesis filament elongation.

Our model envisages that a peak in auxin concentration at the end of meiosis triggers filament elongation and prevents premature anther dehiscence and pollen maturation; the subsequent decline in auxin concentration releases the block and triggers these processes (1). To further test our model we have measured auxin concentration in anthers at different developmental stages and determined the relative role of single TIR, AFB receptors in the different stamen developmental processes.

Cecchetti, V., Altamura, M.M, Falasca, G., Costantino, P. and Cardarelli, M. (2008) Plant Cell, 20, 1760-1774.

P042 PERIANTHIA in proliferation and differentiation of stem cells

Annette T Maier1, Sandra Stehling-Sun1,2, Sarah Schilli1, Jan U Lohmann1,3

1Max-Planck-Institute for Developmental Biology, Tübingen, Germany, 2Whitehead Institute for Biomedical Research, Cambridge MA, USA, 3University of Heidelberg, Department of Stem Cell Biology, Heidelberg, Germany

The transition of stem cells from proliferation to differentiation is one of the most fundamental and important processes during development of multicellular organisms. Here we report that the bZIP transcription factor PERIANTHIA (PAN) plays a key role in controlling this transition in the shoot apical and floral meristems of *Arabidopsis thaliana.* Recently we could show that PAN promotes differentiation of floral stem cells by directly activating the homeotic patterning gene *AGAMOUS (AG)*. Consistently, flowers of *pan* mutants grown in short days display indeterminacy and organ transformation defects similar to weak ag mutant flowers.

Since *PAN* RNA is not confined to flowers, but also is detected in the center of the SAM in a region overlapping with the known stem cell regulators *WUSCHEL (WUS)* and *CLAVATA3 (CLV3)*, we were interested to uncover its function in this tissue. We found that on the one hand *PAN* expression is dependent on the activity *WUS*, while at the same time *WUS* and *CLV3* RNA accumulation is modified in *pan* mutants. Consistently, we identified components of the Cytokinin signaling system, which are known to be important for SAM function, acting downstream of *PAN*. Furthermore, *PAN* seems to be a target of Redox dependent posttranscriptional modification, while at the same time being involved in the transcriptional control of the Redox sensing system.

Thus, *PAN* seems to be embedded into a complex regulatory network with inputs both at the transcriptional, as well as the posttranscriptional level. The redundant nature of the network might have concealed some functions of PAN so far, but combining classical genetics with tools of systems biology might allow us to dissect its important role in plant stem cell control.

P043 Molecular and genetic control of endosperm growth in Arabidopsis

Jing L1, Frederic Berger

1Temasek Life Sciences Laboratory, Singapore

In flowering plants, the seed is produced by a double fertilization event, and is composed of the endosperm and the embryo, surrounded by maternal integuments. The endosperm, which controls the supply of maternally provided nutrients to the embryo, plays a central role in seed size determination. Endosperm growth is regulated by epigenetic pathways such as DNA methylation and parental dosage balance. Mutations in *IKU* genes result in premature arrest of endosperm growth and give rise to small seeds. However, all *IKU* genes are involved in signaling and transcriptional control and we still don't know what are the effectors controlling endosperm and seed size and if there is any crosstalk with epigenetic control. By transcriptome analysis comparing gene expression in seeds from wild type, *iku1* and *iku2* mutants, we found that the expression of 14 genes are altered in both *iku* mutants. Interestingly, we found some of them also changed in small seeds resulting from maternal excess. To further identify the gene network of endosperm growth, by positional cloning, we are undergoing clone an *IKU* independent mutation gene in *chengyu* mutant, where endosperm growth arrests even earlier than *iku* mutants and produces even smaller seeds than that of *haiku* mutant.

P044 The volumetric component of individual leaf expansion: Taking into account sub-epidermal tissues in the description of leaf expansion over time

Nathalie Wuyts1, Genevieve Conejero2, Jean-Luc Verdeil2, Catherine Massonnet1, Jean-Luc Regnard3, Evelyne Costes3, Christine Granier1

1Lepse, IBIP, INRA-SupAgro, Montpellier, France, 2PhiV, UMR DAP, CIRAD, Montpellier, France, 3AFEF, UMR DAP, INRA-SupAgro, Montpellier, France

Most leaf development studies at the cell and organ levels have been limited to the leaf surface, with data referring to the leaf surface area and to the number and surface area of epidermal cells. However, leaf sub-epidermal tissues, the palisade and spongy mesophyll, contain the main actors in photosynthesis. The number and thickness of palisade cell layers and the volume occupied by spongy mesophyll (cells and intercellular spaces) affect the accumulation of photosynthates and, as such, whole plant growth. Studies into the leaf phenotype of growth-affected *Arabidopsis thaliana* mutants have revealed a higher variability in leaf thickness than in leaf surface area. In general, there is no correlation between these two variables, which means that to describe a leaf phenotype, leaf volume has to be taken into account. A method has been developed for high-resolution imaging of leaves in three dimensions using multiphoton laser

scanning microscopy, and for the analysis of images, providing data on volumes and volumetric proportions of cells and tissues and cell density. The method has been used in the study of *A. thaliana* leaf expansion from emergence to the onset of senescence for leaves located at different nodal positions in the rosette, completing our knowledge of individual leaf development processes with its volumetric component. The method will further be applied in the study of leaf plasticity in response to the environment for both *A. thaliana* and apple tree, a model and an agronomic species, respectively.

P045 Probing phenotype and molecular profile reproducibility: A comparison of Arabidopsis leaf growth across ten laboratories

Catherine Massonnet1, AGRON-OMICS Consortium2

1INRA, Montpellier, France, 2www.agron-omics.eu, EU

A major goal of the life sciences is to understand and model how molecular processes control phenotypes. Because the study of biological systems relies on the work of multiple independent research groups, biologists commonly assume that organisms with the same genetic make-up will display similar phenotypes when grown in comparable conditions. We investigated to what extent the growth and molecular phenotypes of identical Arabidopsis genotypes can be reproduced in different laboratories adhering to a standardized protocol. The results were obtained in a pan-European experiment performed in ten locations across six different countries. First, we determined the appropriate environmental conditions and a minimum set of leaf growth variables marking the contrasts between three selected Arabidopsis accessions: Col, Ler and Ws. We then shared a detailed protocol among all laboratories with the aim to assess the reproducibility of leaf phenotype, and of metabolite and transcript profiles extracted from the same leaf samples. The statistical analysis of the data revealed significant differences between measurements obtained from distinct locations, sometimes resulting in a change of genotype ranking in terms of growth performance. Our findings underscore that the challenge of describing, monitoring and precisely controlling environmental conditions is generally underestimated. However, we also demonstrate that independent growth and molecular profile datasets can be used to distinguish between genotypes when produced with particular attention for environmental parameters. This comparative analysis pinpoints likely variables that account for differences in separate laboratories.

P046 CHD3 proteins and Polycomb group proteins antagonistically determine cell identity

Ernst Aichinger1, Corina Belle Villar1, Lars Hennig1, Claudia Köhler1

1Department of Biology and Zurich-Basel Plant Science Center, Swiss Federal Institute of Technology, ETH Centre, CH-8092 Zurich, Switzerland

Transcriptional regulation by changing chromatin states are important mechanisms during differentiation and development. Many enzymes are described to influence the nuclear organization of DNA. First, chromatin modifying enzymes like members of the Polycomb group (PcG) proteins are involved in the regulation of chromatin marks and second, chromatin remodelling enzymes using the energy of ATP hydrolysis are responsible for changes of nucleosome position or composition associated with changes in nucleosomal DNA accessibility. PICKLE (PKL) and PICKLE RELATED (PKR) proteins belong to the CHD family, which is characterized by the presence of tandemly arranged chromodomains. We have investigated the genetic interactions of *pkl* and *pkr* mutants as well as mutants in *PcG* genes and their role in establishing cell identity. Our results let us propose that PKL together with PKR proteins and PcG proteins have antagonistic roles in establishing cell identity in plants.

P047 Definition of the quiescent center in the root meristem of Arabidopsis adventitious roots

Federica Della Rovere, GiuSeppina Falasca, Laura Fattorini, Maria Madalena AltaMmura

Dipartimento Biologia Vegetale, Sapienza Universite di Roma, Rome, Italy

Adventitious roots (ARs) are essential for the root system in a lot of plants. In Arabidopsis one/two ARs are present at the hypocotyl base, and their formation is increased by exogenous/endogenous auxin. Moreover, the stem superficial tissues (thin cell layers, TCLs) produce ARs *in vitro* in the presence of auxin (IBA, 10 μM) and cytokinin (Kin, 0.1μM). Our aim was to investigate on the definition of the quiescent center (QC) in the ARs. We also investigated whether an auxin maximum precedes AR initiation in competent cells, and where it localizes in the AR primordium meristem. We used two QC marker lines previously tested in primary and lateral roots (i.e., *QC25* and *pAGL42:GFP*) and a *DR5:GUS* line (harbouring uidA gene driven by the auxin-inducible DR5 promoter). The seedlings were grown either under hormone-free conditions or in the presence of 2μM NAA, or of IBA (10μM) plus Kin (0.1μM). Under the latter condition, we also investigated QC definition in ARs from TCLs cultured *in vitro*. The results *in planta* show that *DR5:GUS* activity precedes AR formation from hypocotyl competent cells, and this occurs independently on the exogenous hormones. However, IBA+Kin was optimal for improving AR formation *in planta,* whereas NAA caused conspicuous callus and frequently anomalous ARs. Expression of QC markers was observed in QC-positioned cells, and in surrounding cells, of the protruding AR primordia, however it was also observed in not-regularly shaped QCs, e.g. in those of twin apices and in callus superficial regions (NAA treatment). The QC markers expression was coupled with DR5:GUS activity in the AR meristem, both *in planta* and in TCLs. The results point to a clear relationship of auxin with the definition of QC in ARs. Moreover, they show that QC markers/auxin maximum may be also expressed in the absence of regular pattern formation in the root meristem.

P048 Functional characterization of membrane proteins expressed in the Arabidopsis phloem

Rozenn Le Hir1, Dipankar Chakraborti1, Mathieu Castelaim1, Peter Immerzeel1, Thomas Moritz1, Sylvie Dinant2, Catherine Bellini1,2

1UPSC, Dpt of Forest Genetics and Plant Physiology, SLU, 90183 Umea, Sweden, 2IJPB, INRA Center, 78026 Versailles, France

From several phloem transcriptome databasis we identified 6 ABC transporters from the ABCG subgroup and 3 AtN3 nodulins. We confirm their phloem expression during plant development and characterized KO mutants and over-expressing lines.

AtABCG11 was recently shown to be required for cuticular wax export.[1-4] Nevertheless, a knockout mutation leads to pleiotropic phenotypes, which fits with a more pleiotropic expression pattern and a broader role. The metabolomic characterization that we performed indicates that the mutant is affected in different aspects of lipid metabolism including BR biosynthesis. We have identified 3 additional uncharacterized *ABCG* genes that are expressed in the phloem. We make the hypothesis that AtABCG11, can heterodimerize with them and possibly transport different intermediate compounds in lipid biosynthesis pathways.

N3 type nodulines are also ubiquitous transmembrane proteins and are suspected to be involved in signaling.[5,6] We identified 3 members of this family that are expressed in the phloem. KO mutants show a decrease in biomass when grown in long days but not in short days, suggesting a role in photosynthates partitioning or nutrition in general. Experiments are currently in progress to further understand the role of these proteins.

1 Bird, D., *et al* (2007) Plant J. 52, 485-498
2 Luo, B., *et al* (2007) Plant Cell Physiol. 48, 1790-1802
3 Panikashvili, D., *et al* (2007) Plant Physiol. 145, 1345-1360
4 Ukitsu, H., *et al* (2007) Plant Cell Physiol. 48, 1524-1533
5 Guan, Y.F., *et al* (2008) Plant Physiol. 147, 852-863
6 Yang, B., *et al* (2006) Proc. Natl. Acad. Sci. U. S. A. 103, 10503-10508

P049 Regulation of KNOTTED1 cell-to-cell trafficking by a chaperonin protein

Xianfeng (Morgan) Xu, Jing Wang, Yoselin Benitez, David Jackson

Cold Spring Harbor Laboratory, NY, USA

Cell-to-cell communication plays critical roles in specifying cell fate and coordinating development in multi-cellular organisms. A new paradigm for such communication in plants is the selective trafficking of transcription factors through plasmodesmata (PDs), channels that traverse the cell wall and connect all plant cells. We have taken an unbiased genetic strategy to dissect the mechanism of PD trafficking.

The maize KNOTTED1 (KN1) homeodomain protein was the first plant protein found to selectively traffic through PD, and its trafficking appears to be important for its function in stem cell maintenance. A gain-of-function trafficking assay in Arabidopsis was developed to demonstrate that the KN1 homeodomain is necessary and sufficient for trafficking *in vivo*. This system provides a simple and tractable model to understand how proteins traffic and to isolate mutants defective in trafficking.

As a proof of concept for our strategy, a mutant with attenuated KN1 trafficking has been identified as a chaperonin gene. This chaperonin appears essential for PD trafficking of some but all non-cell-autonomous proteins, and biochemical evidence suggests a physical association between chaperonin and KN1. Proteins are thought to undergo partial unfolding during PD translocation, which makes the discovery of this chaperonin particularly exciting. A functional characterization of chaperonins, the first ever factor so far known to be critical for KN1 PD trafficking will further our understanding of developmental regulation and mechanisms of selective cell-to-cell trafficking. In addition, it may give mechanistic insights into this elaborate protein folding machinery, which is not well understood in any system at a molecular level.

P050 BLADE-ON-PETIOLE1 and 2 control Arabidopsis leaf morphogenesis through regulation of *YABBY* and *KNOX* genes

Chan Man Ha1,2, Ji Hyung Jun1,2, Jennifer Fletcher1,2

1Plant Gene Expression Center, USDA/UC Berkeley, 800 Buchanan Street, Albany, CA 94710, USA 2Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA 94720, USA

Lateral organs including leaves are derived from the shoot apical meristem (SAM). Class 1 *KNOTTED-LIKE HOMEOBOX (KNOX)* genes play key roles in promoting SAM activity, and their expression is largely excluded from lateral organ primordia. Ectopic *KNOX* gene activation during Arabidopsis lateral organ formation causes severe morphological defects, highlighting the importance of correctly regulating *KNOX* gene expression. In Arabidopsis, *BLADE-ON-PETIOLE1 (BOP1), BOP2*, and several *YABBY (YAB)* genes are among the factors that negatively regulate *KNOX* gene expression in lateral organs. Here, we show that ectopic blade development in *bop1 bop2* leaves is strongly suppressed by *YAB* gene mutations in a dosage-dependant manner, and that three *KNOX* genes also make more modest contributions to *bop1 bop2* ectopic leaf blade formation. However, the combination of *yab* mutations with knox mutations restores nearly wild-type leaf morphogenesis to *bop1 bop2* plants at the morphological and cellular levels. Taken together, our data reveal unexpectedly complex interactions between *BOP, KNOX* and *YAB* gene activities during leaf morphogenesis.

P051 BLADE-ON-PETIOLE (BOP1) and BOP2 regulate Arabidopsis leaf morphogenesis by directly controlling *AS2* expression along the proximal-distal axis

Ji Hyung Jun1,2, Chan Man Ha1,2, Jennifer Fletcher1,2

1Plant Gene Expression Center, USDA/UC Berkeley, 800 Buchanan Street, Albany, CA 94710, USA, 2Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA 94720, USA

Separation of meristem cell fate from lateral organ fate in the Arabidopsis shoot apex is established by the

antagonistic relationship between class I *KNOTTED-LIKE HOMEOBOX (KNOX)* gene activity and *ASYMMETRIC LEAVES1 (AS1)* and *AS2* gene activity. However, the molecular mechanisms that activate *AS1* and *AS2* expression specifically in organ primordia are still unknown. *BOP1* and *BOP2* encode BTB/POZ and ankyrin repeat-containing proteins that control leaf morphogenesis by negatively regulating class I *KNOX* gene expression in the petiole region. Here we report that BOP1 is a direct upstream regulator of *AS2* during leaf development by demonstrating that BOP1 binds to the *AS2* promoter around bZIP binding sequences. *BOP* activity is specifically required for *AS2* induction in the proximal region of developing cotyledons and rosette leaves, and *AS2* expression driven by the *BOP1* promoter complements the *bop* mutant phenotypes. We also show that *bop1 bop2* mutants restore the capacity for shoot meristem formation in *stm* mutants by failing to initiate ectopic *AS2* expression in the shoot apex. Finally, BOP1 and BOP2 function in the nucleus and have the capability to function as transcriptional coactivators when recruited to target gene promoters. Taken together, our data indicate that BOP1 and BOP2 control leaf morphogenesis along the proximal-distal axis by directly regulating proximal *AS2* expression, establishing the conditions for repression of class I *KNOX* genes in cells adjacent to the meristem-organ boundary to promote the separation and patterning of lateral organ primordia.

P052 *DORNROESCHEN* and *DORNROESCHEN-LIKE* function with the *CUC* genes and MP in modulating embryo symmetry via auxin-dependent pathways

John Chandler, Melanie Cole, Petra Comelli, Wolfgang Werr

Cologne University, Cologne, Germany

A fundamental phase of Arabidopsis embryogenesis comprises the transition of a radially symmetrical globular embryo to a bilaterally symmetrical heart stage embryo, a change wrought by cotyledon development and boundary separation.

The AP2 domain paralogues *DORNROESCHEN (DRN)* and *DORNROESCHEN-LIKE (DRNL)* redundantly control embryo and cotyledon development. Both genes also control floral organ development. The transition from radial to bilateral symmetry also involves auxin signalling and response and redundant functions of the *CUP-SHAPED COTYLEDON (CUC)* genes. Double mutants between *drn* or *drnl* and individual *cuc* mutants show a large increase in penetrance of cotyledon defects, which suggests the genes in each case act redundantly and/or additively. Triple mutants between *drn drnl* and *cuc* mutants show almost complete phenotypic penetrance, implying that these genes are almost sufficient for cotyledon specification. An asymmetry in *CUC* and *STM* gene expression is seen in *drn* and *drn drnl* double mutants, showing that *DRN* and *DRNL* are upstream of and regulate the spatial expression these genes.

We have shown that *DRN* is downstream of auxin responses involving *MONOPTEROS (MP)* in cotyledon tips, by several approaches, including mutation of auxin response elements in the *DRN* promoter, expression of *DRN::GFP* in a *mp* background and by ChIP. *DRN* also functions upstream of auxin signalling/perception/response as shown by *DR5::GFP* and *PIN1* expression.

Genetic interactions between *drn* or *drnl* and genes involved in local auxin biosynthesis and polar auxin transport will be presented and these allow the separation of *DRN* and *DNRL* functions and the basis of their redundancy to be dissected. Redundancy has also been addressed and demonstrated via promoter swap experiments of *DRN* or *DRNL*, driving *DRNL* or *DRN* expression, respectively, and complementation of *drnl* mutants.

P053 Could the extent of cell division, cell expansion and endoreduplication in a leaf be controlled by leaf expansion itself?

Catherine Massonnet, Sébastien Tisné, Juliette Fabre, Nathalie Wuyts, Denis Vile, Myriam Dauzat, Christine Granier

INRA, Montpellier, France

Leaf area expansion is affected by many environmental conditions including incident light, soil water content, and day-length. At the cellular level, these changes are associated with differences in cell number and/or cell size, but also with differences in the extent of endoreduplication. The functional relationships between cellular processes and leaf area expansion have been evaluated by mutational analysis and the study of transgenic lines. A few studies have shown that the regulation of leaf size could be disrupted by alterations in genes involved in cell division, cell expansion or endoreduplication, but many attempts to increase leaf size by modifying cell division or expansion have failed. A multi-scale high-throughput phenotyping and modelling approach was used in our group to determine how these cellular processes interact with the regulation of leaf area expansion both in collections of accessions, populations of recombinant inbred lines and selected mutants affected either in endoreduplication, in cell cycle regulation or in cell expansion. Both the quantitative genetics and statistical modelling approaches lead to the conclusion that these three cellular processes are controlled, at least to some extent, by whole leaf and whole plant developmental processes. As a consequence, their impact on leaf growth itself is expected to be limited which is consistent with many experimental results.

P054 Analysis of the impact of cellular processes on leaf growth of *Arabidopsis thaliana* by a high-throughput mutant approach

Catherine Massonnet, Alexandra Tinaut, Denis Vile, Juliette Fabre, Nathalie Wuyts, Alexis Bediée, Christine Granier

INRA, Montpellier, France

Leaf growth is a dynamic process in 3D that results from a combination of two key cellular processes : cell division

and cell expansion. The mechanisms underlying the control and coordination of these two processes are not well identified. In this study, we propose to use a high-throughput mutant approach to try to elucidate the role played by these cell processes in leaf expansion both in area and thickness. One hundred mutants affected either in cell cycle regulation, cell wall properties, organisation of the cytoskeleton or endoreduplication processes were grown under similar controlled environmental conditions in the PHENOPSIS phenotyping platform. Leaf growth was analysed in area and thickness at different organizational levels, such as cells, the individual leaf and the whole plant.

Among the 100 mutants, 76 did not modify significantly their leaf growth compared to the wild-type neither in area nor in thickness. The remaining 24 mutants were affected either in their leaf area, or leaf thickness or both. There was no relationship between leaf area and leaf thickness within the 100 mutants. Leaf thickness depended more on the proportion of lacuna parenchyma tissue, which was highly negatively correlated to the proportion of palisade parenchyma tissue. Our analysis show an independent control of leaf area and leaf thickness during leaf growth and revealed the necessity to consider these two variables independently in the perspective of a dynamic 3D leaf growth model.

P055 Comprehensive mapping of *ARF* expression reveals novel auxin responses in the plant embryo

Eike H Rademacher, Barbara Möller, Annemarie S Lokerse, Dolf Weijers

Laboratory of Biochemistry, Wageningen University, Wageningen, the Netherlands

A multitude of developmental processes is regulated by auxin-dependent signalling events, which are mostly mediated by changes in gene expression. Auxin controls gene expression by releasing *ARFs* from inhibition by Aux/IAA proteins. Hence, *ARFs* are the executors of auxin-dependent transcription and therefore form the pivotal point in translating auxin signals into transcriptional responses.

Analysis of single and double *ARF* knock-out mutants has demonstrated redundant functions. However the phenotypes observed so far can only account for part of the auxin responses that occur during plant development. Further redundancies between *ARFs* are plausible, but the comprehensive unguided generation of multiple knock-out lines among the 23 *ARF* genes is unrealistic.

We reasoned that only those *ARFs* that are co-expressed in a cell would be able to mediate local auxin responses. Therefore we generated transcriptional fusions of all *ARF* promoters to a sensitive nuclear GFP reporter and determined the respective expression patterns at cellular resolution in the developing embryo. We found 7 *ARFs* to be expressed in various cell types of globular embryos. Surprisingly, many of these *ARFs* are expressed in the extra-embryonic suspensor, and we find that inhibition of *ARFs* in this cell type leads to a transformation to embryo identity. We are currently generating higher order mutant combinations of the respective *ARFs*, and will present our latest results. Interestingly, we find that each distinct cell type has a unique set of *ARFs*. When swapping promoters between an “embryo” *ARF* and a “suspensor” *ARF*, we find that development is strongly impaired, suggesting that *ARFs* are not generally interchangeable. Our systematic *ARF* expression analysis has uncovered new cell-specific auxin response machineries, whose existence could explain how auxin can trigger different responses in distinct cell types.

P056 Molecular control of secondary growth initiation in the Arabidopsis shoot

Silvia Herold1, Eva Maria Sehr1, Raffael Lichtenberger1, Jiri Friml2, Javier Agusti1, Thomas Greb1

1Gregor Mendel Institute of Molecular Plant Biology, 2VIB / University of Ghent

Lateral expansion of growth axes is essential for land plants to create extended shoot and root systems. Lateral or secondary growth is mediated by the cambium, a two-dimensional meristematic tissue, which is organised as a cylinder enclosing the centre of growth axes. *Arabidopsis thaliana*, like most dicotyledonous plants, generates a continuous vascular cambium in the shoot by establishing meristematic activity between primary vascular bundles in the so called interfascicular regions. Surprisingly, in spite of its significance for plant growth and the accumulation of biomass, knowledge about the molecular control of secondary growth initiation is very limited.

Here, we study the initiation of the interfascicular cambium (IC) as a hallmark of secondary growth initiation in Arabidopsis shoots. Genetic and pharmacological approaches show that, in addition to auxin, strigolactone (SL) signalling promotes IC formation and thus adds secondary growth to the list of SL responses. Based on reporter gene analysis, we observe a gradual reduction of *PIN3* and *PIN7* expression along the shoot toward the shoot base suggesting that auxin accumulation and secondary growth initiation depend on a reduction in auxin transport capacity. The PIN expression gradient is, however, not altered in SL-deficient backgrounds, arguing that SLs promote secondary growth independently of auxin transport capacity regulation. In addition, tissue-specific transcriptional profiling of interfascicular regions during IC formation has revealed candidate genes that could act as integrators of long-distance signalling essential for the establishment of cambium identity in interfascicular regions.

P057 Targets of PLETHORA, master regulators of Arabidopsis root development

Marijn Luijten, Inez Terpstra, Anja van Dijken, Maartje A Gorte, Guy Wachsmann, Vera Jansweijer, Kalika Prasad, Pankaj Dhonukshe, Ben Scheres, Renze Heidstra

Utrecht University, Utrecht, The Netherlands

A clade of four *PLETHORA (PLT)* homologues is necessary for root formation, acting largely in an additive and dosage dependent manner. Promoter activity and protein

fusions of *PLT* homologues display gradient distributions with maxima in the stem cell area strongly correlating with a transcriptional auxin response maximum. PLT protein dosage appears to be translated into distinct cellular responses, with high levels promoting stem cell activity and maintenance; lower levels promote mitotic activity of stem cell daughters; and further reduction is required for cell differentiation. Upon induced ectopic expression of *PLT2*, roots are produced from the shoot apex, which together with the loss-of-function data coins *PLT* genes are master switches for root development.

Previous experimental data and modeling revealed a regulatory loop whereby *PLT* patterning genes become restricted in response to a PIN-mediated auxin maximum to define the root primordium during embryogenesis and, in turn, start controlling root-specific PIN expression to stabilize the auxin maximum.

To investigate the molecular role of *PLT* genes in this loop and to characterize the different responses to *PLT* gene activities, microarray analysis was performed to identify direct and indirect targets of PLT proteins. Chromatin IP was used to confirm PLT binding to target promoters. Target gene expression is in progress to assess how much of the response is due to PLT concentration effects. A main outcome from the PLT target analysis sofar not only reveals the transcriptional feedback towards auxin transport but also points to extensive feedback control regulating auxin biosynthesis and signaling, positioning the *PLT* genes in a regulatory knot.

P058 The multifaceted role of Lon protease in organelle biogenesis and post-germinative growth of *Arabidopsis thaliana*

Stamatis Rigas1, Gerasimos Daras1, Costantinos Fasseas1, Lee Sweetlove2, Polydefkis Hatzopoulos1

1 Department of Agricultural Biotechnology; Agricultural University of Athens; Athens, Greece, 2Department of Plant Sciences; University of Oxford; Oxford UK

While the transcription, translation, import and processing of nuclear encoded mitochondrial proteins has received considerable attention in plants, very little is known about the role of proteases in protein stability, turnover and assembly within mitochondria. Members of the ATP-dependent proteases predominantly maintain quality control of proteins in eukaryotic organelles. A genetic screen led to the identification of Arabidopsis *Lon1* protease mutants that exhibit a post-embryonic growth retardation phenotype. Translational fusion to YFP revealed Lon1 subcellular localization in plant mitochondria. Lon1 is highly expressed in rapid growing plant organs and in inflorescence to fulfill the high energy requirements. Mitochondria isolated from *lon1* mutants had a lower capacity for respiration via complexes II and IV and the activity of TCA cycle key enzymes was reduced. Heterologous *Lon1* expression complemented the respiratory deficient phenotype of the yeast *PIM1* gene homolog. The morphology of mutant mitochondria is impaired and reminiscent to the promitochondria morphology in dry seeds. Germination efficiency of plants carrying the *lon1* mutant alleles was dramatically diminished when seeds were germinated at elevated temperature. In contrast to the weak *lon1-2* allele, the polypeptide encoded by the strong *lon1-1* allele carries the predicted sensor- and substrate-discrimination domain possibly allowing substrate recognition and binding. This type of molecular recognition hinders further degradation by the complementary Lon-independent proteolytic machineries that could result in an extra deleterious accumulation of protein aggregates into *lon1-1* mitochondria. Designed remodeling of Lon proteases will untangle the function and their multifaceted roles in plant organelle biogenesis and maintenance.

P059 BOB, a new triple fluorescence clonal deletion system

Guy Wachsmann, Ben Scheres, Renze Heidstra

Utrecht University, Utrecht, The Netherlands

One of the most common methods for understanding the function of a gene is by comparing its wild-type and mutant phenotypes. However, specific questions regarding the cell or tissue specific role can be answered only by deleting or restoring the wild-type allele in specific cell types. So far we have been using a two components system for clonal deletion analysis which consists of an inducible *CRE* recombinase T-DNA insertion and a second construct, *pCB1-GENE*, harboring the wild-type complementing allele (under its own promoter) flanked by two tandem *loxP* sites. However, complemented lethal mutants needed to be genotyped after clonal analysis to confirm excision of all wild-type copies. Especially problematic are mutants that cause gametophytic lethality in males and females which hampers crossing or even allele maintenance in a heterozygote condition. In order to circumvent these problems we designed a new clonal deletion system, Brother Of Brainbow (BOB). The wild-type allele is flanked by two pairs of incompatible lox sites (*lox*N and *lox*2272). Starting with a single insertion homozygous plant, each recombination event can excise the complementing wild-type allele by one of the two lox pairs, translocating a 35S promoter 5' to either a CFP or RFP coding sequence. Consequently, simultaneous expression of both CFP and RFP in a given cell indicates a complete deletion of both wild-type copies. We will present data on the implementation of the BOB system to test the role of the gene *RETINOBLASTOMA-LIKE1 (RBR1)* in specific cell types of the root stem cell niche.

P060 The D-type cyclin *CYCD4;1* controls pericycle cell size in the root apical meristem and lateral root density in Arabidopsis

Jeroen Nieuwland1, Spencer Maughan1, Walter Dewitte1, Simon Scofield1, Luis Sanz2, Jim Murray1

1Cardiff University, Cardiff, UK, 2Universidad de Salamanca, Salamanca, Spain

The size and shape of plant cells are important factors in morphogenesis. Cell size can be modulated by the rate of cell division. Here we show that loss-of-function mutations

in the D-type cyclin *CYCD4;1* causes fewer but larger cells in the pericycle of the root meristem. Promoter-reporter fusions show that *CYCD4;1* expression occurs in the pericycle cells located at protoxylem poles of the meristem, potentially the future sites of lateral root initiation. The *cycd4;1* mutant shows a reduction in lateral root density without affecting primary root growth. Low levels of ectopic auxin restore both pericycle cell sizes and lateral root density in the mutant but the induction of supernumerary lateral root production in response to auxin is not impaired in *cycd4;1*, suggesting that *CYCD4;1* is required for the normal spacing of laterals but not the formation of new lateral roots in response to exogenous auxin. Consistent with this, no expression of *CYCD4;1* or up-regulation in response to auxin was observed in the mature pericycle. Furthermore, we show that *CYCD4;1* expression is dependent on sucrose and that low sucrose levels applied to wt mimics the *cycd4;1* phenotype, both on lateral root numbers and pericycle cell size in the basal meristem. This indicates that *CYCD4;1* is rate-limiting in sucrose-dependent lateral root formation. We conclude that *CYCD4;1* regulates meristem pericycle cell size and affects lateral root density in Arabidopsis.

P061 Redundancy of CVP2 and CVL1 reveals a link between phosphoinositide signaling and the ARF GAP, SFC/VAN3 in establishment of foliar vein patterns

Francine Carland, Timothy Nelson

Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA

COTYLEDON VASCULAR PATTERN2 (CVP2) is an inositol polyphosphate 5' phosphatase (5PTase) involved in the regulation of foliar vein patterning in Arabidopsis foliar organs. 5PTases are known to contribute to animal and yeast cell polarizing events by providing phosphoinositide (PI) lipids for appropriate targeting of lipid binding proteins during vesicle trafficking. In contrast to the closed vein pattern in wild type foliar organs, *cvp2* mutants have an increase in free vein endings and a resulting open vein network. Plants with mutations in both *CVP2* and its close relative *CVP2LIKE1 (CVL1)* have a loss in vein cell polarity resulting in a discontinuous vein pattern identical to that of *scarface/vascular network defective3 (sfc/van3)* mutants. *SFC/VAN3* encodes a PH domain-containing ADP-ribosylation factor-guanosine activating protein (ARF-GAP) that likely functions as a regulator of vesicle transport. Given the phenotypic similarities between *cvp2 cvl1* double mutants and *sfc/van3* mutants and because ARF GAPs are often targets of 5PTase activity, we explored the relationship between CVP2, CVL1 and SFC/VAN3. *cvp2 sfc/van3* double mutants show enhanced vein pattern defects and *CVP2*, *CVL1* and *SFC/VAN3* have significantly overlapping expression patterns in developing vascular cells, providing additional support that the genes reside in the same pathway. Furthermore, we demonstrate that CVP2 and CVL1 generate the specific PI binding partner for the PH domain of SFC/VAN3. We propose that CVP2 and CVP2LIKE1-mediated phosphoinositide signaling regulates SFC ARF-GAP activity in the maintenance of vascular continuity by providing the lipid ligand that presumably targets SFC/VAN3 to its appropriate subcellular site.

P062 Stem cells, epigenetics and root regeneration

Giovanni Sena1, Xiaoning Wang1, Hsiao-Yun Liu1, Pui-Leng Ip1, Hugo Hofhuis2, Kenneth D Birnbaum1

1New York University, New York (NY), U.S., 2Utrecht University, Utrecht, The Netherlands

Regeneration is the re-establishment of cellular identities and patterns in damaged organs. In plants, indeterminate growth during post-embryonic development is sustained by the activity of stem cell niches in apical and lateral meristems. While it is often assumed that organ regeneration in plants depends on such stem cell niches, an alternative hypothesis is that it differs in this respect from indeterminate growth.

We use the Arabidopsis root to investigate the role of the stem cell niche during plant organ regeneration, by integrating over time confocal imaging with global transcriptional profiling of roots after complete whole-tip excision, in various genetic and chemical backgrounds. This gives us an unprecedented view on the change in cell-specific character and morphology during regeneration.

Our results suggest a rapid restoration of missing cell fate and function before the recovery of stem cell activity. Surprisingly, mutants deficient in stem cell niche maintenance were still able to re-establish the lost pattern and cell fates, indicating that a functional niche is not required for organ reorganization as it is for indeterminate growth.

Recently, we began to study the role of epigenetic regulation of the cellular competence to participate to regeneration. Interestingly, a mutant of the Polycomb-like group in Arabidopsis, exhibiting reduced H3K27 histone methylation activity, showed a significant increase of such competence.

Finally, to analyze the contribution of single tissues to the root-tip reorganization and to probe the possibility of true cellular trans-differentiation during regeneration, we started an analysis of clonal sectors in our system.

It appears then that fundamental aspects of organ regeneration in plants depend on a combination of cell fate plasticity and patterning mechanisms, independently of stem cell niche activity but influenced by cellular epigenetic state regulation.

P063 The SHR/SCR pathway directly activates genes involved in asymmetric cell division in the Arabidopsis root

Rosangela Sozzani1, Miguel Moreno-Risueno1, Wolfgang Busch1, Jaimie Van Norman1, Teva Vernoux1,2, Siobhan Brady1,3, Walter Dewitte4, Jim Murray4, Philip Benfey1

1Duke University, Durham, NC, USA, 2ENS, Lyon, France, 3UC Davis, Davis, USA, 4Cardiff University, Cardiff, UK

The combined activity of two transcription factors, SHORT-ROOT (SHR) and SCARECROW (SCR), is required for asymmetric cell division of the cortex/

endodermal initials (CEI) in the Arabidopsis root. To begin to understand the dynamics of the SHR/SCR regulatory network, we employed a conditional activation system. Inducible versions of both SHR and SCR were placed in the background of a ground-tissue specific marker. We used cell sorting combined with microarray time course experiments to examine the transcriptional effects of SHR and SCR specifically in the ground tissue. We identified induction of transcription factor and signaling activity as early responses indicating the presence of a regulatory cascade. Later responses were highly enriched for genes involved in cell cycle progression and mitotic cell division. Among these was a D-type cyclin that we show to be a direct target of both SHR and SCR and expressed specifically in the CEI/CEID cells. Mutation of this gene caused a delay in CEID cell division, in addition to defects in formation of middle cortex. Other mitotic genes activated by the SHR/SCR pathway were also strongly expressed in CEI/CEID cells and their ectopic expression caused additional asymmetric cell division in the ground tissue. These results indicate that SHR and SCR are key regulators of mitotic cell cycle genes within the CEI providing evidence for a direct mechanistic link between development and cell cycle progression.

P064 Genomic dynamics in a cyclin mutant: Limited progressive duplications and a rapid decay

Yixing Wang1, Ajay Jha1, Rujin Chen2, John Doonan3, Ming Yang1

1Oklahoma State University, Stillwater, OK, USA, 2The S. R. Noble Foundation, Ardmore, OK, USA, 3The John Innes Center, Norwich, UK

Production of viable gametes with an unreduced genome due to a meiotic mutation is thought to be a major mechanism for genome duplication and species evolution in nature. However, this scenario remains unexplored because no such a mutant with robust fertility has been found. We here report a null allele of the *CYCLIN A1;2* gene, also named *tardy asynchronous meiosis-2 (tam-2)*, as such a mutant. Meiocytes in *tam-2* underwent only meiosis I but homozygous *tam-2* plants segregated from a heterozygous parent appeared to have normal fertility. We monitored the ploidy levels of *tam-2* plants in four consecutive generations by flow cytometric analysis, starting from the progeny of the heterozygous parents. The first-generation *tam-2* plants were diploid but in the third-generation they had become octoploid, indicating that both the male and female gametes were not reduced in ploidy level for two consecutive generations. Intriguingly, the majority of the *tam-2* plants in the fourth generation had various reduced ploidy levels, and the rest remained octoploid. The same phenomenon was consistently observed in separate lineages from the first-generation *tam-2* plants. These results demonstrate that the dynamics of the mutant genome follows a predictable path of limited progressive duplications and a subsequent decay. This finding may provide an insight into how organisms in nature generally maintain relatively low ploidy levels through a very long evolutionary history. The drastic changes in ploidy and genomic composition over several generations might also provide sufficient time for the organisms to settle down on new stable genomic structures potentially leading to new species formation.

P065 From Arabidopsis to crop plants-conserved transcriptional features of shoot apical meristem

Prem Bhalla, Annie Wong, Dacheng Liang, Farzad Haerizadeh, Mohan Singh

Plant Molecular Biology and Biotechnology, ARC Centre of Excellence for Integrative Legume Research, The University of Melbourne, Vic 3010. Australia

Arabidopsis is an established model system for addressing genetic basis of shoot apical meristem (SAM) formation and maintenance in plant. However, translation of knowledge obtained from Arabidopsis to crop plant species requires thorough investigation of shared and distinct gene expression programs. Among crop plants legumes are particularly important due to their unique developmental and metabolic features such as pod development, symbiotic nitrogen fixation in root nodules that develop from *de novo* differentiated nodule meristems. Accordingly, we have addressed molecular nature of legume SAMs through investigating transcriptional programs of pea and soybean meristems by identifying mRNAs and non-coding small RNAs expressed in SAM. We have sequenced more than 12K transcripts from pea meristem-expressed genes and used custom-designed oligonucleotide arrays to map the transcriptional repertoires of pea SAMs in comparison with non-meristematic tissues. For soybean, we used gene chip arrays to map SAM gene expression programs in meristems at vegetative stage and during their transition to floral meristems. Analysis of the data indicated the striking occurrence of abiotic stress-related transcripts, including trehalose metabolism genes, in SAMs during the early floral transition process. Our data indicates that molecular events mediated by multiple hormonal pathways are part of the regulatory mechanisms controlling the floral transition and flowering process in soybean. Comparison of transcriptional programs of pea and soybean SAMs with available data on maize and Arabidopsis systems have revealed transcriptional programs conserved in plant shoot apical meristems as well as those unique to legume systems. The data from cross species comparison of shoot apical meristem gene expression programs point toward key role of epigenetic chromatin remodelling factors in stem cell maintenance. Overall, our data on conserved SAM transcriptional signatures provide unique resource for dissecting gene regulatory networks by loss of function approaches.

P066 The last common ancestor of ferns and seed plants contained all three clades of the *WOX* gene family including a WUS/WOX5 ancestor

Judith Nardmann, Pascal Reisewitz, Wolfgang Werr

Institute of Developmental Biology, University of Cologne, 50923 Cologne

The Arabidopsis genome encodes 15 members of the Wuschel-related homeobox *(WOX)* gene family, with two prominent members, *WUSCHEL (WUS)* and *WOX5*,

involved in stem cell homeostasis in the shoot and the root meristems, respectively. Phylogenetic analysis based on homeodomain (HD) protein sequences divides the WOX pedigree into three major branches: An ancient branch containing Arabidopsis WOX13-related sequences present in all land plants and some green algae, a second clade of WOX8/9/11/12 homologues in vascular plants and a modern clade (WOX1-7/WUS) already present in ferns.

We examined the origin of the modern branch in two basal angiosperms *(Amborella trichopoda, Nymphaea jamesoniana)*, three gymnosperms *(Pinus sylvestris, Ginkgo biloba, Gnetum gnemon)* and one fern *(Ceratopteris richardii).* Our analysis shows that all members of the modern clade exist at the base of the angiosperm lineage, including WUS and WOX5 orthologues.

In contrast, all three gymnosperm genomes each encode a single WUS/WOX5 homologue, consistent with a monophyletic origin of the root and shoot stem cell-promoting function and suggesting sub-functionalisation in the course of angiosperm evolution. In further support of this, the WUS/WOX5 pro-orthologues are expressed in gymnosperm shoots and roots. Other modern clade members in the gymnosperm lineages are WOX2, WOX3 and WOX4.

So far, we could identify five WOX homologues in *Ceratopteris richardii:* two WOX13 homologues, two which group to the WOX9/11 clade and one which is placed into the modern clade, indicating that all three WOX family clades were already present in the last common ancestor of pteridophytes and seed plants.

Currently, the function of the isolated genes is being addressed via the analysis of cellular expression patterns and by complementation experiments.

P067 FPA controls pre-mRNA 3' end site selection

Csaba Hornyik1, Lionel Terzi2, Katarzyna Rataj2, Jacqueline Marshall2, Gordon Simpson1,2

1SCRI, Dundee, Scotland, 2Dundee University, Dundee, Scotland

FPA functions within the autonomous pathway to control expression of the floral repressor, FLC. In addition, FPA is required for RNAi and the regulation of targets of RNA mediated chromatin silencing. Other components of the autonomous pathway share these functions, but there appears to be a degree of redundancy in the targets they control.

FPA encodes a protein with three RNA recognition motifs, raising the possibility that it functions through binding to RNA. However, the mechanism by which FPA functions in flowering time control and RNA silencing is unclear.

We have discovered that FPA controls poly(A) site selection in its own pre-mRNA. FPA promotes selection of a promoter proximal site within intron1, leading to the formation an alternative mRNA isoform that would code for a severely truncated protein. This negative autoregulation of FPA is highly reminiscent of the mechanism by which a second RNA binding protein within the autonomous pathway, FCA, functions. However, we have discovered that FCA and FPA mediate regulated 3' end formation in a genetically independent manner.

Our findings identify a molecular activity for FPA, provide a molecular explanation for the redundancy of FCA and FPA function and implicate alternative RNA 3' end formation as a key regulatory level at which gene expression and RNA silencing is controlled.

P068 Investigating the role of Tre6P in the shoot apex of *Arabidopsis thaliana*

Vanessa Wahl1, Markus Schmid2, Luise H Brand2, Regina Feil1, Ursula Krause1, Mark Stitt1, John E Lunn

1Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany, 2Max Planck Institute for Developmental Biology, Tuebingen, Germany

Trehalose-6-phophate (Tre6P) is the intermediate of trehalose synthesis. It has been implicated in sugar-signalling pathways in plants in several recent publications. In particular, Tre6P appears to be a signal of sucrose status, since changes in sucrose lead to parallel changes in Tre6P levels.[1] In *Zea mays* Tre6P was suggested to play a role as a mobile short-range signal to regulate meristem development.[1] Tre6P is produced by trehalose-6-phosphate synthase, encoded by the *TPS1* gene. *TPS1* overexpressing plants are very small and have lanceolated leaves and sterile flowers. Null *tps1* mutants are embryo lethal,[3] whereas knockdown of *TPS1* by means of an artificial microRNA gives rise to plants that are much bigger than wildtype. We have investigated *TPS1* expression by RNA *in situ* hybridization in various aerial tissues throughout *Arabidopsis thaliana* development. *TPS1* transcript is present in the vasculature and in the peripheral zone of the vegetative meristem. We are currently investigating the effect of cell specific manipulation of trehalose metabolism on stem cell formation, maintenance and differentiation. Together with Tre6P measurements in apices of wildtype and meristem mutant plants (e.g. stip), our results will give insight into how a given Tre6P signal is integrated into the complex network regulating meristem function.

1 Lunn *et al*, Biochemical Journal (2006) 397, 13
2 Satoh-Nagasawa *et al*, Nature (2006) 441, 227
3 Eastmond *et al*, The Plant Journal (2002) 29, 225

P069 *MINIYO* links transcriptional elongation to stem cell progeny differentiation

Maite Sanmartín1, Michael Sauer1, Alfonso Muñoz1, Jan Zouhar1, Angel Ordóñez1, Wilhelmina van de Ven1, Elena Caro2, María Paz Sánchez2, Natasha V Raikhel 3, Crisanto Gutiérrez2, José J Sánchez-Serrano1, Enrique Rojo1

1Centro Nacional de Biotecnologia CSIC, Madrid, Spain, 2CBM, CSIC-UAM, Madrid, Spain, 3Center for Plant Cell Biology UC Riverside, USA

In animal stem cells (SC), transcription is initiated in the majority of genes, including silenced developmental

regulators. Productive transcriptional elongation, however, is limited to a minimal set of genes. Reactivating stalled RNA Polymerase II to resume elongation of developmental regulators has emerged as a possible mechanism to trigger differentiation in metazoans, but genetic proof for this hypothesis is lacking. To study SC fate regulation, we searched for Arabidopsis mutants affected in meristem development. The *miniyo (myo)* mutant shows delayed and defective onset of differentiation in all SC niches of the plant. Conversely, *MYO* overexpression induces premature differentiation of SC progeny, indicating that *MYO* is a key factor regulating this fate switch. *MYO* mRNA is mainly present in meristems, while nuclear accumulation of a *MYO-GFP* fusion protein is restricted to cells in the meristem periphery, suggesting additional mechanisms direct MYO activity to SC progeny differentiation sites. MYO interacts with RNA Polymerase II and is required for the transcription of key developmental regulators driving differentiation in both shoot and root meristems. Moreover, MYO positively regulates transcriptional elongation, and this activity is required for its role in inducing differentiation. Our results establish MYO as a key factor to drive differentiation of SC descendants in plants. Thus, the function of MYO as transcriptional elongation factor reveals surprising similarities between plants and animals in the control of the SC/differentiation fate switch.

P070 Characterization of CDPKs in flower onset and timing

Julia Legen1, Susanne Matschi1, Joachim Kurth1, Fiona C Robertson2, Helen Briggs2, Alex A R Webb2, Tina Romeis1

1Freie Universitaet, Berlin, Germany, Department of Plant Biochemistry, 2Cambridge University, UK, Department of Plant Sciences

Flowering in Arabidopsis is regulated via genetic and environmental stimuli. A genetic component that regulates flower development is Flowering Locus T (FT). It has been suggested that FT together with Flowering Locus D (FD) leads to the activation of *APETALA 1 (AP1)* thereby initiating flower formation. We have identified Calcium Dependent Protein Kinases (CDPK) that may participate in flower initiation and development. CDPKs are characterized by four different domains: N terminal Variable domain, Kinase domain responsible for phosphate transfer, a Junction domain with autoinhibitory activity and C terminally located four EF motifs having the capacity to bind free calcium (Ca^{2+}). In particular, two mutant alleles of an AtCPK isoform show phenotypic characteristics of a late flowering plant, identifying this CDPK as a flowering promoter under certain light conditions. Physiological and genetic evidence obtained by crosses of the *cpk* mutant line with gibberellic acid (GA) pathway mutants or mutants related to photoperiodic pathway, such as *elf3-1, spa1-7*, *phyB* and 35S:CO, respectively, suggest that this CDPK functions independent of GA pathway and is a true photoperiodic protein. Furthermore, the analysis of the circadian oscillation pattern of cytosolic free Ca^{2+} based on the aequorin reporter gene crosses in *cpk* mutant positions it downstream of the circadian clock. Interestingly, the gene expression patterns of major transcription factors in flowering are unaltered with exception of *AP1*, whose expression is delayed. Biochemical characterization of CDPK revealed a calcium dependent autophosphorylation activity. The function of CDPK autophosphorylation as well as substrate phosphorylation of factors involved in flower development is currently under investigation.

P071 Cutting to the chase: Using laser-capture microdissection and transcript profiling to reveal biological insights about Arabidopsis seed development

Ryan Kirkbride1, Mark Belmonte1, Sandra Stone1, Julie Pelletier1, Jiong Fei1, Meryl Hashimoto1, Anhthu Bui2, Brandon Le2, Chen Cheng2, Min Chen2, Jungim Hur2, Robert Goldberg2, John Harada1

1University of California, Davis, CA, USA, 2University of California, Los Angeles, CA, USA

The Arabidopsis seed is a complex structure consisting of both maternal and zygotic tissues of varying ploidy. Throughout seed development massive changes occur, ranging from embryo progression from a single-celled zygote to fully patterned embryo, to endosperm proliferation and differentiation into three distinct regions, or seed coat maturation and senescence. Detailed analysis of transcript populations within the different parts of the developing Arabidopsis seed has been severely hampered by the seed's small size, accessibility of compartments and small amount of material. Using laser-capture microdissection coupled with Affymetrix GeneChip hybridization analyses, we have profiled the RNA populations of seven distinct regions of the seed across five developmental stages, ranging from the pre-globular to mature-green stages of seed development. These datasets combine both positional and temporal information into the most comprehensive study of seed transcript populations to date. This new information is being used to reevaluate and refine our picture of known developmental processes that operate within the seed, such as hormone production and storage reserve accumulation. Analyses of these datasets are also uncovering new biological insights into the function of under-characterized compartments like the three distinct endosperm domains. Spatiotemporal differences in transcript abundance as it pertains to hormone production, carbon metabolism, storage reserve accumulation will be discussed.

P072 Production of superoxide in the Arabidopsis root tip is the result of photomorphogenesis

Irina Strizh, Andrey Buglak

Faculty of Biology, M.V. Lomonosov Moscow State University, Moscow, Russia

Light and reactive oxygen species (ROS) are well known crucial factors that regulate plant growth and development. The intriguing topic is whether these factors interact during the plant development. It is known that superoxide is necessary for the root hairs tip growth and for the root elongation in Arabidopsis. These data typically

obtained by studying mechanisms of the root growth in the completely illuminated plants. We suggested that mechanisms of the root growth and development in the dark may differ from those established for the light-grown plants. In 5 days-old Arabidopsis seedlings, grown in sterile conditions under 16/8 h photoperiod the typically root growth zones dependent nitroblue tetrazolium (NBT) distribution could be easily detected. NBT – is a widely used indicator, which forms a dark blue formazan precipitate in contact with superoxide. Surprisingly, we have not found NBT staining of the root elongation zone in the dark-grown seedlings. CuSO4 that typically result in increasing in the levels of ROS induce extensive root tip NBT staining in dark-grown seedlings. We propose that there is no superoxide production in the dark-grown Arabidopsis root tip. However, slight NBT staining was detectable in the region with the high density of the root hairs in the dark-grown plants. We suggest that different enzymes are responsible for the superoxide generation in the root hairs and in the cells of root elongation zone. We have shown that deetiolation of seedlings under white light cause the NBT staining of the root tip. The dark-grown seedlings exhibit extensive NBT staining of the root tip if kinetin in various concentrations was present in the growth medium. NBT staining was also observed in the root tip of seedlings grown in the dark in the presence of sodium nitroprusside - an NO donor that is known as a stimulator molecule in plant photomorphogenesis. We propose that photomorphogenetic changes result in the superoxide production in the root elongation zone in Arabidopsis.

P073 Analyzing a novel root cell elongation regulator, UP BEAT1

Hironaka Tsukagoshi1, Siobhan Brady2, Philip Benfey1

1Duke university, Durham, NC, USA, 2University of California Davis, Davis, CA, USA

In plants, stem cell centers known as meristems are located at the tip of the root and shoot. The root meristem generates cells that divide several times before entering a zone of rapid elongation without division (elongation zone) after which they go on to differentiate. The process of cell expansion dramatically increases the volume of the cell, often as much as 100 fold.

Both root meristem size and root growth are regulated systematically as the rates of cell division and elongation are synchronized. However the molecular details of the transcription network regulating rapid root cell elongation are poorly understood. To identify the transcriptional networks regulating rapid root cell elongation, we used our RootMap, which comprises high-resolution gene expression profiles obtained from fine sections along the developmental axis of the Arabidopsis root. We chose several transcription factors that show a peak of gene expression at the boundary between the root meristem zone and elongation zone. We then screened Arabidopsis T-DNA insertion mutants for these transcription factors. Preliminary characterization of one of these, *UPBEAT1* has shown that downregulation results in a larger plant while upregulation results in a smaller plant.

The molecular and cytological characterization of the *upbeat1* mutant should provide new insights into the molecular interactions controlling rapid cell expansion. Here we report the molecular mechanisms of meristem maintenance that were regulated by *UPBEAT1*.

P074 MicroRNA-regulated SBP-box genes control fertility in Arabidopsis

Shuping Xing, Vanessa Quodt, Susanne Höhmann, Nora Bujdoso, Arne Grande, Maria Salinas, Rita Berndtgen, Heinz Saedler, Peter Huijser

Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany

SBP-box genes represent plant specific transcription factors associated with developmental processes such as the vegetative to reproductive phase transition and microsporogenesis. Eleven out of the seventeen SBP-box genes in Arabidopsis, known as *SPL* genes, are targeted by the microRNA156/157 *(miR156/157)*. Constitutive over-expression of *MIR156 (MIR156OX)* in wild type results in down-regulation of its target genes and causes a delayed vegetative to reproductive growth transition. Whereas the temporal expression of these eleven *SPL* genes correlates strongly with (the switch to) reproductive growth, that of the non-targeted *SPL* genes does not. The one exception is *SPL8*, its mRNA does not have a *miR156/157* target site but is specifically expressed at the reproductive stage and its function is required for proper anther development. Mutation of *SPL8* reduces the number of pollen sacs formed and the pollen produced. Remarkably, anthers from *MIR156OX* transgenics also occasionally produce fewer pollen sacs like observed for *spl8* mutants. We therefore constructed *MIR156OX spl8* double mutant plants and found these to be fully sterile. Anthers were affected at a very early stage of development and completely lacked pollen sacs. Manual pollination using wild type pollen suggested that the female organs of these double mutants were also affected. In addition, double, triple and quadruple loss-of-function mutants between *spl8* and the *miR156* targeted *SPL* genes all showed further enhancement of the *spl8* phenotype. Finally, we used *miR156* target mimicry to elevate *miR156* targeted *SPL* gene expression levels against a *spl8* mutant background and observed a rescue of the *spl8* mutant phenotype. Our experimental data strongly suggests that *SPL8* and *miR156* targeted *SPL* genes act in a redundant manner to secure both male and female fertility in Arabidopsis.

P075 Integrating the genetic and physical maps of Arabidopsis: Identification of mapped alleles of cloned essential *(EMB)* genes

David Meinke, Colleen Sweeney, Rosanna Muralla

Oklahoma State University, Stillwater, OK, USA

More than 130 genes with an embryo-defective (*emb*) knockout phenotype are included on the classical genetic map of Arabidopsis. Many of these genes remain to be cloned. Hundreds of additional *EMB* genes have been

cloned and catalogued (www.SeedGenes.org) but not mapped. To facilitate *EMB* gene identification and assess the level of saturation for essential genes in Arabidopsis, we updated the classical map, compared the physical and genetic locations of all mapped loci, and performed allelism tests between mapped (but not cloned) and cloned (but not mapped) *emb* mutants with similar map locations. Initial efforts focused on chromosomes 1 and 5. Two hundred pairwise combinations were tested and more than 1100 total crosses were screened. Sixteen of 51 mapped emb mutants examined were found to be disrupted in a known *EMB* gene. Informative alleles of a wide range of published *EMB* genes *(YDA, GLA1, TIL1, AtASP38, AtDEK1, EMB506, DG1, OEP80)* were discovered. Two EMS mutants isolated 30 years ago, T-DNA mutants with complex insertion sites, and a mutant with an atypical, embryo-specific phenotype were resolved. The frequency of allelism encountered was consistent with past estimates of 750 to 1000 *EMB* loci. New *EMB* genes identified among mapped insertion mutants included *CHC1*, which is required for chromatin remodeling, and the Arabidopsis ortholog *(SHS1)* of the maize *BRITTLE1* locus required for normal endosperm development. The alignment of genetic and physical maps presented here should facilitate the continued analysis of essential genes in Arabidopsis and further characterization of a broad spectrum of mutant phenotypes in a model plant.

P076 Analysis of the Arabidopsis homologs of the LST8 protein, a conserved partner of the TOR kinase complex

Manon Moreau1, Gilles Clément1, Marianne Azzopardi1, Christophe Robaglia2, Christian Meyer1

1Unite de Nutrition Azotee des Plantes, Institut Jean-Pierre Bourgin, INRA Versailles, 78026 Versailles Cedex, France, 2Laboratoire de Genetique et Biophysique des plantes, UMR 6191 CNRS-CEA-Universite de la Mediterrane, Faculte des Sciences de Luminy, 163 Avenue de Luminy, 13009 Marseille Cedex 9, France

The Target of Rapamycin (TOR) kinase is present in all eukaryotic organisms. In yeast and animal cells, TOR is a well known key regulator of cell growth, controlling translation, transcription, ribosome biogenesis, autophagy, cell cycle, nutrient import, and actin cytoskeleton organization in response to environmental signals and energy status. However, its role remains poorly understood in plants. In yeast and mammals, TOR functions in two multiprotein complexes, which have different roles in cells. TORC1 is composed of TOR, RAPTOR and LST8 (or GβL) while TORC2 is formed by TOR, LST8 and RICTOR proteins. Homologs of TOR complexes proteins have been identified in Arabidopsis and previous studies have shown that tor or raptor mutations are embryo lethal. Moreover we have also already shown that the level of expression of the Arabidopsis TOR gene correlates with plant growth.

In order to investigate the nature of the TOR complexes and the role of the various putative TOR partners, we studied the putative homologs of the *Lst8* genes in Arabidopsis. LST8 is a 35kDa protein containing WD40 domains that is thought to be involved in the TOR complexes stability and activity. In yeast, LST8 is a negative regulator of amino acids synthesis genes via Rtg1/3 transcription factors and is also implicated in vesicular trafficking and retrograde signalling. We will present evidence that one of the two Arabidopsis *Lst8* genes can complement yeast *lst8* mutants. Study of homozygous *lst8* mutant plants revealed drastic growth retardation, default in leaves development, bushy phenotype, very reduced flowering, and lack of viable seeds. Moreover *lst8* mutant plants accumulated amino acids and showed many metabolic changes.

P077 *EARLY IN SHORT DAYS 7 (ESD7)* encodes the catalytic subunit of the DNA polymerase epsilon and is required for flowering repression through a mechanism involving epigenetic gene silencing

Ivan del Olmo, Leticia Lopez-Gonzalez, Manuel A Pineiro, Jose A Jarillo

Centro de Biotecnologia y Genomica de Plantas, Madrid, Spain

We have characterized a mutation affecting the Arabidopsis *ESD7* gene encoding the catalytic subunit of the DNA polymerase epsilon, AtPOL2A. In other organisms this enzyme has been involved in diverse processes such as DNA replication and repair, chromatin remodelling and transcriptional silencing. *esd7-1* mutation causes early flowering independently of photoperiod, shortened inflorescence internodes and altered leaf and root development. *esd7-1* is a hypomorphic allele whereas KO alleles displayed an embryo-lethal phenotype, suggesting that this gene is essential for the proper embryo development and viability. The SAM and the RAM in the *esd7-1* seedlings were found to exhibit an altered disposition that might correlate with the abnormal expression pattern of SAM and RAM marker genes.

ESD7/AtPOL2A is expressed ubiquitously at low levels in all the tissues analyzed and its expression is up-regulated by genotoxic stress. In fact, *esd7-1* showed higher sensitivity to UV-C light and to mitomycin than wild type plants and altered expression of genes involved in DNA repair mechanisms by homologous recombination such as *RAD51, BRCA1, BRCA2* and *GR1*. *esd7* early flowering phenotype requires functional FT and SOC1 proteins and might be related to the deregulation of *AG* and *AG*-like gene expression found in *esd7-1*. Loci involved in the modulation of the chromatin structure dynamic, such as *TFL2* and *EBS*, which also negatively regulate *FT* expression, were found to interact genetically with *ESD7*. Moreover, *fasciata 2 (fas2)* mutations suppressed *esd7-1* early flowering phenotype and *INCURVATA 2(ICU2)* was found to be epistatic to *ESD7*. Discrete regions of the chromatin of *FT* and *AG* loci were enriched in activating epigenetic marks in the *esd7-1* mutant. We concluded that *ESD7* might be participating in processes involved in chromatin-mediated cellular memory.

P078 *Expression of SHORT INTERNODES/ STYLISH* family genes in auxin biosynthesis zones of aerial organs is dependent of a GCC-box-like regulatory element

Magnus Eklund1, Izabela Cierlik1, Veronika Ståldal1, John Chandler2, Eva Sundberg1

1Department of Plant Biology and Forest Genetics, Uppsala BioCenter SLU, Box 7080,SE-750 07, Uppsala, Sweden, 2Institute of Developmental Biology, University of Cologne,Gyrhofstrasse 17,D-50923, Cologne, Germany

Although auxin biosynthesis in *Arabidopsis thaliana* has been intensively studied there are only a few proteins acting in this process that have been so far identified, among them are members of the *SHORT INTERNODES/STYLISH (SHI/STY)* gene family. In previous work we have shown that overexpression of *STYLISH1 (STY1)* leads to increased IAA levels and IAA biosynthesis rates, and additionally, the *sty1 sty2* double mutant has reduced IAA levels. Furthermore, we have been able to show that *STY1* acts as a transcriptional activator of genes controlling several nodes of tryptophan-dependent auxin biosynthesis.

Here, we present a study on the transcriptional regulation of *SHI/STY* family members that will further help us to gain an insight into transcriptional regulation of auxin biosynthesis.

We attempted to modulate the normal expression pattern of *STY1* by mutating a putative regulatory element, a GCC-box, in the proximal promoter region and conserved in most *SHI/STY* genes in Arabidopsis. Mutations in the GCC-box abolish all expression in aerial organs of the adult plant, however, the expression in the lateral root primordia, hypocotyls and proximal cotyledon regions was not altered. We also show that induction of the transcriptional activator *DORNRÖSCHEN-LIKE (DRNL)* activates transcription of *STY1* and other *SHI/STY* family members and that this activation is dependent on a functional GCC-box. However, *STY1* expression in the *drnl drn* double mutant, carrying knock-down mutations in both DRNL and its close paralogue *DORNRÖSCHEN (DRN)*, was not significantly altered, suggesting that several other genes might have functions redundant with *DRN/DRNL*, or that the activation of *STY1* only occurs when *DRNL* is ectopically expressed.

In current studies we are analyzing new putative candidate genes that may act as upstream regulators of *STY1* gene and therefore regulate auxin biosynthesis.

P079 Arabidopsis leaf phenomics

José Manuel Pérez-Pérez1, Silvia Rubio-Díaz1, Diana Hernández-Romero1, Joaquín Sánchez-Soriano2, Stijn Dhondt3, Gerrit Beemster3, María Rosa Ponce1, José Luis Micol1

1Division de Genetica and Instituto de Bioingenieria, Universidad Miguel Hernandez, Campus de Elche, 03202 Elche, Spain, 2Centro de Investigacion Operativa, Universidad Miguel Hernandez, Campus de Elche, 03202 Elche, Spain, 3VIB Department of Plant Systems Biology, Ghent University, Gent, Belgium

Leaves are lateral determined organs mainly devoted to photosynthesis, which are also important for nutrient storage, defence and stress responses. The final shape and size of a leaf result from a tight coordination between cell proliferation and cell expansion. Although an organ-size checkpoint controlling leaf size remains to be identified, several *Arabidopsis thaliana* mutants impaired in cell proliferation display compensated cell enlargement. To genetically dissect the mechanisms underlying plant organ growth, we have analyzed a collection of 111 leaf mutants. We are using leaf size perturbation as a phenotypic trait indicating loss of function in a gene involved in organ growth. The quantization of several size and shape parameters at the organ, tissue and cellular levels in these mutants has helped us to establish a numeric framework for leaf parameters that would be useful for the characterization of leaf phenotypes in indexed collections of loss-of-function mutants. We and others have positionally cloned several of these genes, whose products participate in a variety of processes, such as polar cell expansion, transduction of hormonal signals, gene regulation, plastid biogenesis, and chromatin remodeling.

P080 14-3-3 proteins and their importance for plant development

Katrin Weckermann, Claudia Oecking

ZMBP, Tübingen, Germany

The Arabidopsis 14-3-3 family can be divided into two major phylogenetic groups, the epsilon-group and the nonepsilon-group, the latter consisting of three organizational subgroups. 14-3-3 proteins are involved in several signal transduction pathways by binding to pS/pT motifs in a sequence specific manner, thereby inducing a change in the activity state of the respective target protein. The amino acid residues involved in binding of the phosphorylated consensus motif are highly conserved among all isoforms, which would suggest that they exhibit similar functional properties. However, the N- and C-termini are nearly unique to each isoform. Consequently, the question arises as to whether particular 14-3-3 isoforms have distinct biological functions. Transgenic plants characterized by T-DNA induced loss-of-function alleles of individual 14-3-3 non-epsilon isoforms do not show an obvious phenotype. We therefore assume that members of particular phylogenetic subgroups are functionally redundant. Remarkably, up to now, we were unable to isolate knock out allels for any of the 14-3-3 epsilon-group member. Hence, transgenic lines which should allow ethanol-inducible RNA interference based gene silencing of the 14-3-3 isoforms constituting the epsilon group were generated. The specific and efficient reduction of the expression of these 14-3-3 homolgs gives rise to a severe developmental delay, finally resulting in lethality. Seedlings are characterized by a disorganized root tip, defect in root hair as well as lateral root formation – processes known to be regulated by auxin. Consequently, these growth defects can be complemented by auxin application. Additionally, the roots do not show a gravitropic response which may be indicative of an impaired auxin transport. Taken together these results indicate that either functional redundancy between members of the epsilon- and the nonepsilon-group does not exist or that

generation of this phenotype is dosage-dependent. To gain further insights, we are currently trying to overexpress a nontargeted 14-3-3 isoform in the RNAi lines.

P081 New interaction partners for 14-3-3 proteins

Marion Grieser, Stephan Müller, Claudia Oecking
ZMBP Tübingen, Germany

Members of the eukaryotic 14-3-3 family are highly conserved proteins that have been implicated in the regulation of distinct biological processes and pathways by protein-protein interactions. Most notably, 14-3-3 proteins bind to phosphoserine/phosohothreonine motifs in a sequence-specific manner and are required to induce a change in the activity state of the respective target protein. 14-3-3s associate to form homo- or heterodimers with a saddle-shaped structure, with each monomer forming an extended groove that allows binding of the phosphorylated consensus interaction motifs (R/K(X)XXpS/TXP). Arabidopsis harbors thirteen 14-3-3 isoforms, most of the functions of which are currently unknown.

This project aims at identifying proteins that interact with selected 14-3-3 isoforms or isoform subgroups. By means of the yeast-two-hybrid system we screened the isoform kappa as a 'bait' against a 'prey'-cDNA library obtained from etiolated seedlings.

According to the screen performed, a total of 511 proteins have been identified as putative interaction partners. Notably 30% of these targets are involved in signalling, 47.5 % play a role in metabolic processes and further 9% participate in transcription and translation. It is beyond the scope of this project to validate all putative 14-3-3 targets, and therefore we focused on specific signalling proteins. One of them belongs to the JAZ-protein family, the members of which are known as key regulators of jasmonate signalling1. Another putative interaction partner is NPH3, which is essential for PHOT1-dependent phototropic responses in Arabidopsis.

We could show that NPH3 interacts with 14-3-3s in an isoform-independent manner. Interestingly, this interaction is strongly mediated by the C-terminal region of NPH3, which also seems to be required for binding PHOT12.

The interactions will be verified by means of bimolecular fluorescence complementation (BiFC). Furthermore, we will focus on the identification of a 14-3-3 binding motif and the analysis of the biological relevance of significant interactions.

P082 Identifying miRNA regulatory networks in the Arabidopsis root

Natalie Breakfield, Juthamas Sae-Seaw, Jalean Petricka, David Corcoran, Molly Megraw, Xuan Ding, Philip Benfey
Duke University, Durham, NC, USA

It is hypothesized that each cell type has a distinct microRNA (miRNA) expression profile that controls developmental processes in a stage specific manner. Attempts to identify the small RNA populations have been complicated by the presence of multiple cell types and developmental stages within a tissue. The intersection of two technologies, namely cell sorting and high throughput DNA sequencing, are revealing small RNA populations in individual cell types. We are using new sequencing technologies to query the RNA species in sorted populations of specific cell types in the root to identify controls for developmental patterning. Differential expression patterns of known miRNAs in the different cell types have provided clues about which miRNAs are important in root development. Additionally, putative novel miRNAs have been identified and are in the process of being validated. Future work will focus on elucidating the functions of these known and novel miRNAs in the regulation of root development.

P083 Hormonal effect on root regeneration competence

Lihua Shen, Giovanni Sena, Xiaoning Wang, Pui-Leng Ip, Kenneth Birnbaum
New York University, Department of Biology, Center for Genomics and Systems Biology, 100 Washington Sq. East, New York, NY, 10003, USA

The relatively simple layout and ability to regenerate makes Arabidopsis root a good model system for studying organ regeneration. We have recently shown that a regeneration competent zone exists in Arabidopsis root tip, i.e. the root can only regenerate after excision within a certain distance from the root tip. Importantly, we have also shown that root regeneration proceeds even in the absence of an active stem cell niche. Here we ask what factors determine the regeneration competence of the root.

Spatially, the root meristem and regeneration competent zone are both located proximal to the root tip. Functionally, the root meristem contains cells with differentiation potential, making it a likely source for regeneration. Recently, it has been shown that root meristem size decreases when treated with cytokinin. We have tested the effect of cytokinin on regeneration competence. Here we show that the regeneration competent zone is located within the meristem, and that cytokinin, in addition to decreasing meristem size, reduces regeneration competence. We further measured the effect cytokinin has on cell length. We have found that cytokinin does not change cell length within the regeneration competent zone, but does change it above the competent zone. This finding suggests that signals from above the competent zone may affect regeneration competence. Recently, it has been shown that 30nM IAA treatment causes a decrease in elongation zone size, without changing meristem size. Here we show that 30nM IAA treatment reduces regeneration competence in the root, without changing meristem size. Importantly, this evidence suggests that regeneration competence may not be determined by meristem size, as one might expect.

P084 The plant specific BPC/BBR family of GAGA-repeat binding proteins

Ulrich Bloss, Mareike L Hohenstatt, Sabine Hummel, Klaus Harter, Dierk Wanke
ZMBP/Plant Physiology, 72076 Tuebingen, Germany

BPC/BBR proteins comprise a novel class of transcription factors that are confined to the plant kingdom.

BPC/BBR-proteins have been identified due to their specific binding to a conserved element with its simple sequence repeat consensus of 7 fold *(GA/TC)* or higher. BPC proteins in *Arabidopsis thaliana* have properties of animal GAGA-binding factors, but they exhibit no sequence homologies to *Trl* and *Psq* of Drosophila, which encode functionally analogous proteins.

By structural means, the BPCs of *Arabidopsis thaliana* can be subdivided into distinct groups based upon their N-terminal domain. Similarly, phylogenetic analysis based solely on the DNA-binding domain sequence strongly supports the division into the same groups.

So far, three distinct regions could be identified common to most BPC proteins: A N-terminal putative activation domain, a nuclear localization sequence (NLS) and a highly conserved basic DNA-binding domain, which is structured as a typical zinc-finger-like motif at its C-terminus.

The N-terminal putative activation domain is predicted to form a coiled-coil structure. As coiled-coils are known to mediate oligomerization, we investigated the ability of BPCs to form dimers via a Yeast-two-hybrid assay and Bimolecular Fluorescence complementation.

P085 Floral transition in shoot apical meristem of soyabean

Annie Wong, Mohan Singh, Prem Bhalla

Plant Molecular Biology and Biotechnology Laboratory, ARC Centre of Excellence for Integrative Legume Research, The University of Melbourne, Parkville, Victoria 3010

The development of the shoot apical meristem (SAM) into floral meristem is a major event in a plant's life that has to be precisely timed to ensure reproductive success. One key environmental factor that regulates flowering is the change in day length ie photoperiod, as flowering can be controlled by exposure to long days (LDs) or short days (SDs) depending on the plant species. Studies in the facultative LD model plant *Arabidopsis thaliana* have revealed some key elements of the LD pathway. Whether the basic flowering pathways as revealed from studies in Arabidopsis are conserved in soybean, how the regulation is modified to adjust to the growth habit of a vernalisation-unresponsive SD species such as soybean remain to be determined. Our study aims to characterize the molecular events leading to floral transition in soybean SAM. Our approach involves the use of Affymetrix GeneChip Soybean Array containing probe sets for 37,500 sequences to obtain the transcript profiles of SAM during floral transition. Analysis of the resulting microarray data revealed that a total of 331 transcripts have differential expression profiles during the floral transition time points investigated. Further in silico analysis implicates various molecular processes, especially hormonal pathways, critical to the floral transition during soybean development. A comparison of our study with similar floral initiation data from Arabidopsis reveals a conservation of the Arabidopsis MADS box genes in the flowering process in soybean. While there are conserved features of the floral network between Arabidopsis and soybean, the lack of overlap also implies that there are distinct differences between the two species in the underlying networks regulating the floral transition process.

P086 Small RNAs profiling of the shoot apical meristem

Prem Bhalla1, Annie Wong1, Xiujie Wang2, Jerry Zhao2, Farzad Haerizadeh1, Bernard Carroll3, Mohan Singh1

1Plant Molecular Biology and Biotechnology Laboratory, ARC Centre of Excellence for Integrative Legume Research, The University of Melbourne, Parkville, Victoria 3010. Australia, 2Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing, 100101, CHINA, 3ARC Centre of Excellence for Integrative Legume Research, The University of Queensland, St. Lucia, Queensland 4072, Australia

Small RNAs (21-24 nucleotides) including microRNAs (miRNAs) and small interfering RNAs (siRNAs) play crucial roles regulating gene expression in higher organisms including plants. However, small RNAs that may play an important role in the regulation and function of the shoot apical meristem (SAM) of important crop plants such as soybean have not been studied. We have approached this by developing two small RNA (sRNA) libraries from soybean leaf or micro-dissected SAM, respectively. Deep sequencing by 454 sequence by synthesis approach resulted in 400,000 putative small RNAs from both libraries. Sequence analysis confirmed the expression of close to 50 conserved miRNAs in soybean. Most importantly, among these conserved miRNAs, 28 of them could only be found in the SAM sRNA library suggesting that these miRNAs are SAM-specific in their expression. In addition, 277 novel candidate miRNAs have also been predicted and with close to one third displaying specific expression in the SAM sRNA library. The putative target genes corresponding to these novel miRNAs have been identified computationally. Predicted target genes include transcription factors but also genes implicated in various developmental and metabolic processes.

P087 The MCM helicase-binding protein ETG1 supports in sister-chromatid arm cohesion necessary for post-replication repair

Naoki Takahashi1,2,3, Veit Schubert4, Tim Lammens1,2, Klaas Vandepoele1,2, Ingo Schubert4, Dirk Inzé1,2, Minami Matsui 3, Lieven De Veylder1,2

1Department of Molecular Genetics, Ghent University, Gent, Belgium, 2Department of Plant Systems Biology, Flanders Institute for Biotechnology, Gent, Belgium, 3Plant Functional Genomics Research Group, RIKEN Plant Science Center, Yokohama, Japan, 4Institute of Plant Genetics and Crop Plant Research (IPK), D-06466 Gatersleben, Germany

Chromatid cohesion holds the replicated sister chromatids together until the onset of anaphase. Cohesion is not only important to facilitate faithful chromosome segregation during mitosis and meiosis, but also aids DNA repair during replication. However, the importance for sister chromatid cohesion during development of multicellular organisms remains elusive. Previously, we identified the minichromosome maintenance (MCM) helicase-binding ETG1 protein of *Arabidopsis thaliana* as a novel evolutionary conserved replication factor being crucial for efficient DNA replication. Here, we demonstrate that the ETG1 protein is required for sister-chromatid cohesion. ETG1 depletion causes a strong upregulation of G2/M-specific genes, reflecting the activation of a cell cycle checkpoint. Sister-chromatid arm

cohesion is impaired in *ETG1*-deficient cells, whereas sister centromere cohesion is normal. The *etg1* and the *ctf18* cohesion mutations synergistically suppress plant growth in correlation with a strong increase in DNA damage and with induction of DNA stress genes. We conclude that the growth inhibition in *ETG1*-deficient plants is due to inefficient DNA repair caused by the loss of sister chromatid cohesion, and demonstrate that establishment of cohesion is essential for proper development of plants suffering DNA stress.

P088 Identification and characterization of suppressors downstream of the IDA signaling pathway in Arabidopsis

Chun-Lin Shi1, Grethe-Elisabeth Stenvik1, Ane Kjersti Vie2, Atle M Bones2, Reidunn B Aalen1, Melinka A Butenko1

1Department of Molecular Biosciences, University of Oslo, NO-0316 Oslo, Norway, 2Department of Biology, Norwegian University of Science and Technology, NO-7491 Trondheim, Norway

Small peptides play an important role in the coordination of plant growth and development. I*NFLORESCENCE DEFICIENT IN ABSCISSION (IDA),* encodes a putative peptide ligand necessary for the regulation of, floral organ abscission in Arabidopsis. We have showed that IDA is dependent on the two receptor-like kinases (RLK) HAESA and HAESA-LIKE 2 to exert its function (Stenvik *et al,* Plant Cell, 2008). We propose that this peptide-signal receptor system induces a signaling cascade that regulates cell-cell separation in floral abscission zones (AZs). To identify proteins acting downstream of the IDA signaling pathway, a screen for plants showing normal floral abscission was performed on EMS mutagenised *ida* C24 seeds. 24 lines showing a complete rescue of the floral abscission defect were detected. Here we present data from one mutant line (49-1) that has a mutation in a known transcription factor (TF). In addition to rescuing the abscission defect of *ida*, line 49-1 shows some of the same floral phenotypes as plants overexpressing IDA, such as premature floral organ abscission, enlarged AZs, pedicel abscission and production of arabinogalactan (AGs). F2 plants from a genetic cross between *ida* C24 and a known allele of line 49-1 show normal floral abscission in double homozygous mutants. *Promoter::GUS* transgenic plants of the gene mutated in line 49-1 shows a comparable but more extensive expression pattern to that of *pIDA::GUS*. These results suggest that the TF mutated in line 49-1 may act as a suppressor of floral organ abscission in Arabidopsis downstream in the IDA signaling pathway.

P089 A transcriptomic approach to identify light quality specific responses during early stages in seedling development

Florian Peschke, Thomas Kretsch

Albert-Ludwigs-Universitat Freiburg, Institut fur Biologie, Botanik, Schanzlestr.1, 79104 Freiburg i. Br., Germany

Light is one of the most important environmental factors controlling plant development To perceive light quality and quantity, plants have evolved a set of at least of three different photoreceptors: red/far-red light sensing phytochromes, UV-A/blue light receptors and yet unidentified UV-B receptors. The photoreceptor systems display diverse and pleiotropic responses throughout their plant's cycle. Via microarray analysis we analyzed the early stages of plant development against light in dark-grown wild-type *Arabidopsis thaliana* seedlings (Col-0) under various light conditions, i.e. red, far-red, blue, UV-A, UV-B, and white light. Plants were harvested after 45 minutes and 4 hours of light irradiation. Our analyses revealed sets of genes specifically induced by distinct light qualities and by combinations of two or more light qualities. Early responsive genes are induced by all applied light treatments without specificity towards any light quality. Clear light effects were observed after 4 hours of irradiation, whereas blue and far-red light induced a similar set of genes to comparable levels. Early induced genes mainly comprise signaling components, whereas late light responsive genes are mainly linked to plastid development. We established a set of marker genes that can be used for rapid analysis of light induced gene expression in etiolated Arabidopsis seedlings.

P090 Senescence in Arabidopsis siliques: Its role in seed development and nutrition

Carol Wagstaff1, Thomas J W Yang2, Erold Namob2, Jerry Roberts2

1University of Reading, Department of Food Biosciences, Reading, Berkshire, UK, 2Division of Plant Sciences, University of Nottingham, Sutton Bonington, Leicestershire, UK

Senescence of plant organs is a genetically controlled process that regulates cell death to facilitate nutrient recovery and recycling and frequently precedes, or is concomitant with, ripening of reproductive structures. In Arabidopsis the seeds are contained within a silique which undergoes a programme of senescence prior to dehiscence. A transcriptional analysis of the silique wall was undertaken to identify changes in gene expression during senescence. The study revealed that the most highly upregulated genes in senescing silique wall tissues encoded seed storage proteins and this finding has led us to investigate the role of the silique wall in seed filling in more detail. We have used mutants with a delayed senescence phenotype and shown that storage protein accumulation can be modulated as a result. Additionally we are investigating the role of *ARR22* as a potential gating mechanism that regulates seed filling in Arabidopsis siliques that have been subjected to a wounding challenge.

P091 Novel interacting factors of CDD complex, a negative regulator of photomorphogenesis

Tomoko Igawa1, Masayuki Fujiwara1, Yoichiro Fukao1, Xing Wang Deng2, Yuki Yanagawa1

1The Plant Science Education Unit, The Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan, 2Department of Molecular, Cellular and Developmental Biology, Yale University, Connecticut, USA

COP/DET/FUS (Constituitive photomorphogenesis/ De-etiolated/Fusca) proteins negatively regulate photomorphogensis in plants. Their proteins are known

to act as regulatory factors of ubiquitination of some transcription factors such as HY5. Target proteins of 26S proteasome are ubiquitinated through ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin ligase (E3), and the ubiquitinated proteins are caught and degraded by 26S proteasome. In this work, we focus on a COP/DET/FUS protein, COP10. COP10 composes of CDD complex together with UV-damaged DNA binding protein 1 (DDB1) and De-etiolated 1 (DET1), and has an ability to enhance E2 activity. Recently, CDD complex was found to interact with Cullin4 and Rbx1. In this meeting, we show novel interacting factors of the CDD core complex.

P092 Studies on the regulatory role of light in leaf primordia initiation

Saiko Yoshida, Cris Kuhlemeier

Institute of Plant Sciences, University of Bern, Bern, Switzerland

Light is one of the major environmental stimuli, which controls growth and development throughout the plant life cycle. Various kinds of light-regulated morphological changes have been known such as photo-/skotomorphogenesis, shade avoidance and phototropism. At the molecular level, it has been known that many genes are regulated by light and dark. Interestingly, our recent finding suggested that light may also have an influence on leaf primordia initiation. We found that the *aux1 lax1 lax2 lax3* quadruple mutant, carrying mutations in auxin influx carrier genes, showed a much stronger phenotype, such as abnormal phyllotactic pattern in short-days condition than in long-days condition.[1] Therefore, in this study, we examined the regulatory role of light in leaf primordia initiation.

By using the tomato shoot apex culture system, we found that shoot apices grown in the dark stopped producing leaf primordia in the presence of sucrose. Dark treatment did not affect the viability of the apices. Furthermore, light reversed the inhibitory effects of darkness and restarted leaf initiation. On the other hand, the apices cultured in the presence of photosynthesis inhibitors and sucrose could produce new leaf primordia. These results suggested that there may be a photosynthesis-independent but light-dependent pathway which is necessary for leaf initiation. Based on these data, the possible relationships between light and leaf initiation are discussed.

1 Bainbridge, K; Guyomarc'h, S; Bayer, E; Swarup, R; Bennett, M; Mandel, T; Kuhlemeier C, (2008) Auxin influx carriers stabilize phyllotactic patterning, Genes & Development 22: 810-8

P093 Functional characterization of the Arabidopsis mutant *calmodulin2*

Michela Landoni, Alessandra De Francesco, Chiara Tonelli

University of Milan, Department of Biomolecular Sciences and Biotechnology, Milan, Italy

Calmodulin (CAM) is an ubiquitous calcium binding protein whose function is to translate the signals, perceived as calcium concentration variations, into the appropriate cellular responses by the interaction and the subsequent regulation of the activity of many different targets.

In Arabidopsis there are 4 isoforms of CAM which are highly similar, encoded by 7 genes, and one possible explanation proposed for the conservation of the *CAM* gene family is that the different genes have acquired different functions (by subfunctionalization or neofunctionalization) so they can play, maybe overlapping but not-identical roles.

To analyze the functions played by CAM genes we screened the gene-trapping collection EXOTIC looking for insertion in this gene family and here we report the characterization of the Arabidopsis mutant *cam2*. Histological characterization and *in vitro* pollen germination revealed that *cam2* pollen shows normal development but a reduced level of germination in comparison with the wild type and genetic analysis showed a reduced transmission of the *cam2* allele through the male gametophyte. The mutant phenotype was completely rescued by genetic complementation in *cam2 35S::CAM2* plants. These results gave a direct evidence of the involvement of a specific *CAM* gene in pollen germination and support the theory of functional non-redundancy of the *CAM* gene family.

P094 Cell-type specfic auxin responses in the Arabidopsis root

Bastiaan Bargmann, Kenneth Birnbaum

New York University, New York, NY, USA

Auxin is a phytohormone crucial to many different aspects of growth and development. One of the key questions in the field of auxin research is how this molecule can elicit its diverse effects in specific cell types, both during development and in response to the environment. Auxin distribution is altered throughout development and in response to external stimuli, inducing changes in growth patterns of the plant. Transcriptional responses to auxin are known to be modulated by families of ARFs (Auxin Response Factors) and Aux/IAAs. Previous cell-type specific gene expression profiling of the Arabidopsis root has demonstrated that these regulators of auxin-induced transcription have distinct cellular expression patterns.

In order to methodically assess how individual cell types respond to auxin, we have generated cell-type specific gene expression profiles utilizing fluorescent marker lines expressing GFP in particular cell types of the Arabidopsis root and Fluorescence Activated Cell Sorting (FACS). Microarray analysis shows that the various assayed cell types have both overlapping and distinct responses to auxin. Auxin responsive genes can be clustered according to their cell-type specific expression profiles. Conversely, inducible expression of dominant negative mutant Aux/IAA isoforms in the marker lines allows us to investigate the effect of auxin signal deprivation on particular cell types.

In addition, we have developed a transient over-expression system that employs multicolor flow-cytometric analysis and FACS, enabling rapid screening of the effect

of candidate transcription factors on reporter gene activation as well as genome-wide transcriptional consequences. As a proof of concept, transient over-expression of individual homologous isoforms of dominant negative mutant Aux/IAAs is shown to effect unique downstream consequences. This system is used to screen for upstream transcription factors regulating cell-type specific auxin-induced gene expression.

P095 Epidermal cell density is regulated by a negative feedback loop involving the secretory signaling peptide EPIDERMAL PATTERNING FACTOR 2

Kenta Hara1, Toshiya Yokoo1, Ryoko Kajita1, Takaaki Onishi1, Kylee M Peterson2, Keiko U Torii 2,3, Tatsuo Kakimoto1

1Biological Sciences, Osaka University, Osaka, Japan, 2Department of Biology, University of Washington, Seattle, WA, USA, 3PROST, JST, Tokyo, Japan

Regulation of the number and placement of cells is critical for proper development of multicellular organisms. During leaf development, a protodermal cell first makes a fate decision of whether or not to be the meristemoid mother cell (MMC), which undergoes asymmetric cell division forming a meristemoid and its sister cell. The MMC-derived stomatal lineage produces two guard cells that constitute a stoma as well as surrounding non-guard cells. We demonstrate that a small secretory peptide, EPIDERMAL PATTERING FACTOR 2 (EPF2), is produced by cells with asymmetric-division competency, including MMC and its early descendants, and non-cell autonomously inhibits cells from adopting the MMC fate. This feedback loop plays a critical role in regulation of epidermal cell density. EPF2 resembles in its amino acid sequence to EPF1, which regulates stomatal spacing. Our promoter swapping experiments revealed that the coding regions of *EPF1* and *EPF2* are, at least in part, responsible for the specific functions of EPF1 and EPF2. Interestingly, however, both EPF1 and EPF2 require common putative receptor components, TMM, ER, ERL1, and ERL2 to function.

P096 Lipid profiling during Arabidopsis reproductive processes

Yuki Nakamura1, Zhi Wei Teo1, Siou Ting Gan1, Guanghou Shui2, Ryota Koizumi3, Hiroyuki Ohta4,5, Markus R Wenk2,6, Toshiro Ito7

1Temasek Life Sciences Laboratory, Singapore, Singapore, 2Centre for Life Sciences, National University of Singapore, Singapore, 3Graduate School of Biosciences and Biotechnology, Tokyo Institute of Technology, Japan, 4Center for Biological Resources and Informatics, Tokyo Institute of Technology, Yokohama, Japan, 5Research Center for the Evolving Earth and Planets, Tokyo Institute of Technology, Yokohama, Japan, 6Department of Biological Sciences, National University of Singapore, Singapore, 7Temasek Life Sciences Laboratory, Singapore

Flowers are unique in unusually high level of certain lipids, such as phosphatidic acid (PA) and phosphoinositides, which play critical roles in plant signal transduction. The importance of lipids in flower development is further suggested by a plenty of examples in which knock-out of lipid-related genes shows abnormal phenotypes in flower development or reproductive processes. In Arabidopsis, however, lipid metabolism in flowers is largely unknown mainly because flowers are too tiny to harvest in bulk according to the developmental stages. To explore the function of lipids in Arabidopsis reproductive process, we performed developmental stage-specific glycerolipid profiling using a system to synchronize flower development. The results revealed transiently high level of PA at early stages of flower development. The *Phosphatidic acid phosphohydrolase 1 / Phosphatidic acid phosphohydrolase 2 (pah1/pah2)* double mutant is defective in two isoforms of PA phosphatase involved in PA metabolism, which shows high level of endogenous PA. The flowers of *pah1/pah2* occasionally (~1%) showed terminal flower phenotypes. Enhancer mutant screening was performed in the *pah1/pah2* background to search for possible interactors, which gave us enhancer 1 of *pah1/pah2* (*eph1/pah1/pah2*) showing a strong terminal flower phenotype. By isolating the single mutant *eph1*, we confirmed that *pah1/pah2* mutation indeed enhances *eph1* phenotypes. These results suggest that PA metabolism is involved in the maintenance of shoot apical meristems and development of floral meristems.

P097 Functional analysis of *LSH* genes that are direct targets of CUC1 transcription factor controlling organ boundary formation

Seiji Takeda, Keiko Hanano, Yuka Tsubakimoto, Ayano Kariya, Satoko Shimizu, Masao Tasaka, Mitsuhiro Aida

NAIST, Nara, Japan

Plant lateral organs are produced from meristem throughout their life. One of the important events in the organ formation is to generate boundary cells that separate meristem and organs. *cup shaped cotyledon 1 (cuc1) cuc2* double mutant is defective in separating organs and meristem, resulting in organ fusion. *CUC1* and *CUC2* encode transcription factors with NAC domain and are expressed in boundary region in many phase of plant life.

To understand how *CUC1* characterizes boundary region, we identified *CUC1* direct target genes by microarray and *in situ* expression analysis. Among these targets, *LIGHT-DEPENDENT SHORT HYPOCOTYLS 3 (LSH3)* and its homolog *LSH4* encode nuclear-localized protein with unknown molecular function. *LSH3* and *LSH4* are expressed at boundary region in a similar pattern with *CUC1*. Over-expression of *LSH4* results in aberrant leaves and extra floral organ formation. Because other *LSH* genes are expressed in different domain of meristem, spatio-temporal control of *LSH* expression is important for plant development.

P098 The ASH1 HOMOLOG 2 (ASHH2) histone H3 methyltransferase is required for ovule and anther development in Arabidopsis

Paul E Grini1, Tage Thorstensen1, Vibeke Alm1, Gema Vizcay-Barrena2, Susanne S Windju1, Zoe A Wilson2, Reidunn B Aalen1

1Department of Molecular Biosciences, University of Oslo, Norway, 2School of Biosciences, University of Nottingham, UK

SET-domain proteins add methyl groups to lysine (K) residues of histone tails, which may function as marks activating or repressing transcription. The ASH1 HOMOLOG 2 (ASHH2) protein of *Arabidopsis thaliana* groups with *Drosophila* ASH1, a positive maintainer of gene expression, and yeast Set2, a histone H3K36 methyltransferase, and has been implicated as a histone H3K4 or H3K36 methyltransferase. *ashh2* mutants display pleiotropic developmental defects, including early flowering. Here we focus on the role of *ASHH2* in plant reproduction, of homeotic changes in floral organ identity and specific effects on the development of the reproductive organs. On the female side, close to 80% of the mature ovules lack embryo sac. On the male side, anthers frequently develop without pollen sacs and where present show specific defects in the tapetum layer. As a result, the number of functional pollen per anther was reduced by up to ~90%. Transcriptional profiling identified more than 600 down-regulated genes in *ashh2* mutant inflorescences, including genes involved in determination of floral organ identity, embryo sac development and anther/pollen development. Currently, there is a discrepancy in the literature on the primary substrate of ASHH2 methylation. We observed a reduction of H3K36 trimethylation (me3) but not H3K4me3 or H3K36me2 in chromatin from selected down-regulated genes. Thus, our analysis strongly suggests that ASHH2 works via H3K36 trimethylation in the regulation of genes essential in reproductive development.

P099 LC-MS-based proteomics of Arabidopsis phloem sap

Zaidah Rahmat1,2, Mark H Bennett1, Colin G N Turnbull1

1Imperial College London, London, UK, 2Universiti Teknologi Malaysia, Johor, Malaysia

We are interested in phloem as a dynamic compartment that transports not only photoassimilates but also many other metabolites and macromolecules, some of which have long-distance signalling functions. Here, we report preliminary characterisation of the phloem sap proteome of *Arabidopsis thaliana*, aiming to provide an essential data resource for future studies of macromolecule transport and signalling. Using samples derived by petiole exudation into EDTA solutions, tryptic digests were generated from total protein extracts or from excised portions of 1D-SDS PAGE separations. Nano-flow liquid chromatography-tandem MS (LC-MS-MS) was used to generate spectra from peptides, allowing conclusive identification of proteins. A total of 1353 peptides was matched from the MS-MS spectra when searched via MASCOT against the Arabidopsis databases. From these peptides, over 150 unique Arabidopsis proteins were identified. Some of these are homologs of proteins previously identified from phloem of Brassica, rice and cucurbits. However, a substantial proportion represent proteins not previously identified from phloem sap, including many with no functional annotations. Of those with functional annotations, the greatest over-representation was for proteins with roles in abiotic and biotic stress responses.

We have also developed a method for targeted analysis of the systemic protein hormone signal, *FLOWERING LOCUS T (FT)*, using LC-MS with Multiple Reaction Monitoring (MRM). FT protein was detected in phloem or leaves of wild type and positive control FT-overexpressing plants, and was confirmed as absent from *ft* null mutant negative controls.

P100 Genetic screen for stem cell regulators

Ta-Fang Lin1, Jinhui Chen2, Alicja Dolzblasz1, Ivo Rieu1, Thomas Laux1

1Institute of Biology III, Albert-Ludwigs-University of Freiburg, Freiburg, Germany, 2Key laboratory of forestry genetics and biotechnology, Nanjing Forestry University, Nanjing, PR China

Throughout their life span plants produce new organs from stem cells that are located in the shoot and root apical meristems. The transcription factor *WUSCHEL* is expressed in a few cells in the centre of the shoot meristem (called the organization center) and non-cell autonomously specifies stem cell identity. To identify novel factors involved in the stem-cell specification pathway we have performed an EMS mutant screen in a weak *wus-7* allele. Our initial characterization of *wus-7* modifier mutants will be presented.

P101 Identification and analysis of second site mutations that suppress the defective phenotypes of the sterol biosynthetic *dry2/sqe1-5* Arabidopsis mutant

Veronica Gonzalez-Doblas1, Vitor Amorim-Silva2, David Pose1, Omar Borsani 3, Herlander Azevedo2, Victoriano Valpuesta1, Rui Manuel Tavares2, Miguel Angel Botella1

1University of Malaga, Spain, 2University of Minho, Braga, Portugal, 3 University of Montevideo, Urugauy

Sterols are isoprenoid-derived lipids that have essential roles in plant growth and development. For example, the plasma membrane sterol content has been shown to modulate the activity of membrane bound proteins. Although our knowledge of plant sterol metabolism has increased recently, key questions still remain to be answered: Do plant sterols play only a structural role or they are also involve in another roles as signaling molecules?

In a search for essential genes involved in plant drought tolerance we identified an Arabidopsis mutant *dry2/sqe1-5 (drought hypersensitive/squalene epoxidase 1)* that is affected in the *Squalene Epoxidase Gene-1 (SQE1)*.[1] Of the three genes that show squalene epoxidase activity, mutations in *SQE1* cause reduced root and hypocotyl elongation, diminished stature and unviable seeds, indicating an essential role of this gene in plant development.

dry2/sqe1-5 shows altered production of Reactive Oxygen Species (ROS). As RHD2 NADPH oxidase is delocalized in roots hairs, we propose that sterols have an essential role in the localization of NADPH oxidases required for ROS regulation.

We set out to identify second-site mutations that abrogated the drought hypersensitivity and defective developmental phenotypes of *dry2/sqe1-5*. A total of 14 independent plant lines showed a significant reversion of phenotype. The ROS defects were abolished in these suppressors supporting a role for sterols in ROS regulation. The physiological and molecular characterization of the suppressors suggest that sterols play a role in signaling. We are now in the process to identify these second-site mutations using map-based-cloning.

Posé, D *et al* (2009). Plant Journal doi:10.1111/j.1365-313X.2009.03849.x

P102 Characterization of *Arabidopsis thaliana* orthologues of GAAP, a Golgi-localized anti-apoptotic protein

Maija E Sierla, Bart J Feys

Imperial College London, UK

Programmed cell death (PCD) is a genetically controlled process that plays an essential role in eukaryotes during development and in response to pathogens and abiotic stress signals. Apoptosis in animals is a form of PCD that has been well characterized, however, core regulators controlling and executing PCD in plants remain largely unknown. GAAPs (Golgi anti-apoptotic protein) are a novel, evolutionarily conserved group of anti-apoptotic proteins (Gubser *et al*, 2007). GAAPs are small integral membrane proteins with six or seven predicted transmembrane domains but no other known functional domains. Human and viral GAAPs have been shown to inhibit apoptosis induced by a variety of pro-apoptotic stimuli, and are thus far the only characterized members of this gene family. There is an apparent expansion of the *GAAP* gene family in plants, with five paralogous genes present in the *Arabidopsis thaliana* genome (*AtGAAP1-5*). This project aims to characterise Arabidopsis GAAP proteins concentrating on their putative role as regulators of PCD. *AtGAAPs* show generally quite distinct expression patterns with *AtGAAP4* and *AtGAAP2* showing the highest transcript abundance based on publicly available microarray data and RT-PCR analysis. *AtGAAP1* and *AtGAAP3* show tissue specificity with elevated expression levels in pollen and stamen, or seeds, respectively. AtGAAP proteins localise mainly to the Golgi following transient expression as YFP fusions in *Nicotiana benthamiana* plants. Knockout mutant plants for all *AtGAAP* genes have been isolated. To address the issue of redundancy, generation of double and triple mutants is under way. The role of AtGAAPs *in planta* is being tested by examining whether loss of AtGAAP function affects sensitivity of the plant to a variety of stress treatments known to induce cell death. Putative role of AtGAAPs as cell death regulators is also being studied in transient transformation system in *Nicotiana benthamiana.*

Gubser C, Bergamaschi D, Hollinshead M, Lu X, van Kuppeveld FJM and Smith GL (2007) A New inhibitor of apoptosis from vaccinia virus and eukaryotes. PLoS PATHOGENS 3 (2): 246-259

P103 Transcriptional regulation of *GIGANTEA*, a circadian-clock regulated flowering time gene in *Arabidopsis thaliana*

Markus C Berns, Frédéric Cremer, Ingmar Bürstel, George Coupland

Max-Planck-Institute for Plant Breeding Research, Carl-von-Linne Weg 10, D-50829 Cologne, Germany

The initiation of flowering is a key step in the life cycle of all higher plants, marking the transition from the vegetative to the reproductive state. This process is regulated by a broad spectrum of environmental and endogenous factors, such as light, temperature and plant hormones. One of the most extensively studied pathways in this framework in the model species *Arabidopsis thaliana* is the photoperiodic flowering pathway. A key player in this pathway is GIGANTEA (GI), a circadian-clock regulated protein that is most abundant in the evening. The precise timing of *GI*-expression in the evening is crucial for it to fulfil its functions during flower initiation and in the circadian clock, raising the question of how *GIGANTEA* itself is transcriptionally regulated.

To address this question we made use of a phylogenetic shadowing approach. We compared the *GIGANTEA* promoter sequence in eight different Brassicaceae species using several bioinformatics tools. This analysis revealed five highly conserved blocks as well as a number of putatively important transcription factor binding sites within these blocks. Among these were three Evening Elements, cis-regulatory elements that are overrepresented in the promoters of evening phased genes and are capable of conferring evening expression in an artificial promoter system. To test the significance of the Evening Elements in the context of the *GIGANTEA* promoter, mutant promoter constructs were generated and cloned upstream of the firefly luciferase open reading frame. These gene fusions were then introduced into *Arabidopsis thaliana* by Agrobacterium-mediated transformation and luminescence of transformed seedlings was analysed using a Top-Count setup.

P104 Analysis of the Arabidopsis 4CL-like *ACYL-CoA SYNTHETASE5* gene and co-expressed genes reveals an ancient biochemical pathway required for pollen development and sporopollenin biosynthesis

Sung Soo Kim1, de Azevedo Souza Clarice1, Stefanie Koch2, Lucie Kienow2, Katja Schneider2, Che Colpitts3, Dae-Yeon Suh3, Erich Kombrink2, Carl J Douglas1

1Department of Botany, University of British Columbia, Vancouver, BC, Canada, 2Max Planck Institue for Plant Breeding Research, Koln, Germany, 3Department of Chemistry & Biochemistry, University of Regina, Regina, SK, Canada

Formation of pollen and spore walls requires the deposition of sporopollenin, a poorly characterized mixed aliphatic/aromatic polymer with ester and ether linkages

that contributes to the protective and tough exine layer. We discovered that the Arabidopsis 4-coumarate:CoA ligase (4CL)-like enzyme Acyl-CoA synthetase5 (ACOS5) is absolutely required for pollen development. An *acos5* mutant is sterile, devoid of visible pollen grains, and lacks sporopollenin or exine. Phylogenetic analysis revealed that *ACOS5* genes are conserved in land plants (eg, poplar, rice, tobacco, and Physcomitrella). *ACOS5* is transiently and exclusively expressed in tapetum cells, and encodes an acyl-CoA synthetase with highest activity against medium chain hydroxy-fatty acids. *In silico* co-expression analyses identified Arabidopsis genes encoding potential enzymes that could work with ACOS5 to generate sporopollenin monomers. Previous studies and our reverse genetic analyses of co-expressed genes such as a *dihydroflavonol-4-reductase-like gene* and *polyketide synthase (PKS)* genes revealed that mutants in these co-expressed genes are also compromised in male fertility and sporopollenin deposition. Phylogenetic analyses showed that these genes are conserved in land plants including Physcomitrella, and the Arabidopsis and Physcomitrella PKS enzymes have similar *in vitro* biochemical activities and could use ACOS5-generated starter molecules to produce polyketides incorporated into sporopollenin. This work illuminates the outlines of an ancient but previously uncharacterized pathway involved in biosynthesis of the monomeric constituents of the sporopollenin polymer, one of the most robust cell wall matrices known in plants.

P105 Functional characterization of a microtubule-associated protein, MAP20, in Arabidopsis and Poplar

Manoj Kumar1, David Ohman1, Tuula Teeri2, Ines Ezcurra2, Totte Niittylä1, Björn Sundberg1

1Umea Plant Science Centre (UPSC), Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences (SLU), SE-901 83 Umea, Sweden, 2Swedish Center for Biomimetic Fiber Engineering, KTH Biotechnology, SE-106 91 Stockholm, Sweden

Cortical microtubules (MTs) are thought to be exerting their control on cellulose microfibrils (MFs) and other developmental aspects of plant biology via a host of different proteins, collectively known as microtubule-associated proteins (MAPs). Recently, Rajangam *et al* (2008) described a MAP in poplar: *PttMAP20*. This gene is highly expressed during the formation of secondary walls of poplar fibers. We are carrying out functional analysis of *PttMAP20* and its closest Arabidopsis homolog: *AtMAP20*. In Arabidopsis, we have identified several T-DNA insertion lines. These lines show a chemical phenotype in secondary xylem of hypocotyls when analysed by FT-IR and pyrolysis GC/MS. Moreover, over-expression of either the poplar or the Arabidopsis *MAP20* gene in Arabidopsis leads to right handed helical twisting of epidermal cell layers in seedlings, supporting its functional binding to cortical microtubules. In addition, we have produced several transgenic poplar RNAi lines with significantly reduced expression of *PttMAP20* were, as well as *35S::PttMAP20* over-expression (OE) lines. These transgenic lines are currently being phenotyped for wood characteristics using a battery of FuncFiber wood phenotyping tools to search for its function in wood development. Preliminary data obtained from FT-IR analysis suggest that a modified expression of PttMAP20 affects mainly relative lignin and sugar amounts.

P106 Spermidine hydroxycinnamoyl transferase (SHT) – a BAHD acyltransferase involved in pollen development

Etienne Grienenberger1, Sebastien Besseau1, Pierrette Geoffroy1, Delphine Debayle2, Michel Legrand1

1Institut de Biologie Moleculaire des Plantes du CNRS, Strasbourg, France, 2Institut de Biologie Moleculaire des Plantes du CNRS - Plateforme Metabolomique, Strasbourg, France

BAHD acyltransferases catalyze the acylation of many plant secondary metabolites. We characterized the function of *At2g19070*, a member of the BAHD gene family of *Arabidopsis thaliana*. The acyltransferase gene was shown to be specifically expressed in anther tapetum cells at the early stages of flower development. The impact of gene repression was studied in RNAi plants and in a KO mutant line containing a T-DNA insertion in *At2g19070* coding region. Immunoblotting with a specific antiserum raised against the recombinant protein was used to evaluate *At2g19070* gene product accumulation in flowers of various Arabidopsis genotypes including the KO and RNAi lines. Metabolic profiling of flower bud tissues demonstrated a positive correlation between the accumulation of acyltransferase protein and the quantities of metabolites that were identified by tandem mass spectrometry as N1,N5,N10 –trihydroxyferuloyl spermidine and N1,N5-dihydroxyferuloyl-N10-sinapoyl spermidine. These products, deposited in pollen coat, can be readily extracted by pollen wash and were shown to be responsible for pollen autofluorescence. The activity of the recombinant enzyme produced in bacteria was assayed with various hydroxycinnamoyl-CoA esters and polyamines as donor and acceptor substrates, respectively. Feruloyl-CoA and spermidine proved the best substrates and therefore the enzyme was named spermidine hydroxycinnamoyl transferase (SHT). One methyltransferase gene (*At1g67990*), co-regulated with SHT during flower development, was shown to participate in the same biosynthetic pathway by analysing the consequences of its repression in RNAi plants and by characterizing the methylation activity of the recombinant enzyme.

P107 Biosynthesis of phenylpropanoid polyamine conjugates in flower buds of *Arabidopsis thaliana*

Christin Fellenberg, Christoph Bötcher, Thomas Vogt

Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany,

Phenylpropanoid polyamine conjugates are a class of secondary metabolites, occuring in a wide range of many plant species. They accumulate in reproductive organs of higher plants and the accumulation is apparently linked to plant fertility. The biosynthesis and exact biological role

of such conjugates during flower development, was unknown. The presence of polyamine conjugates in *Arabidopsis thaliana* has only been recently established in flowers and seeds. Annotation and location of a cation-dependent O-methyltransferase AtTSM1 specifically in the tapetum of young flower buds enables the subsequent identification of several genes with a putative role in phenylpropanoid polyamine conjugate biosynthesis. Based on corresponding *A. thaliana* knockout mutants and real time PCR data, a biosynthetic pathway of these conjugates is proposed. This pathway involves two methylation steps, catalyzed by two different cation-dependent O-methyltransferases, a cytochrome P450 enzyme (*Cyp98A8*) dependent hydroxylation step, and a conjugating acyl transfer performed by a BAHD-like hydroxycinnamate (HCA)-acyltransferase. LC/MS-based acylpolyamine profiling of *cyp98A8* knockout mutants identified new feruloyl- and 4-coumarolylspermidine conjugates in flowers of *A. thaliana*, consistent with a role of this enzyme in formation of the hydroxylation pattern of these conjugates. In contrast, knockouts of CCoAOMT1 and the HCA-transferase lead to a drastic decrease or a complete loss of the major polyamine conjugates identified in the wildtype flower buds. A complex pattern of minor bis- and trisacylspermidine compounds, likely the products of additional HCA-transferases, were identified in wildtype as well as in all RNAi and knockout lines. The implication of these findings for phenylpropanoid polyamine conjugate biosynthesis in flowers of *A. thaliana* is discussed.

P108 Characterization of two *NF-Y* A genes in *Arabidopsis thaliana*

Monica Fornari, Valentina Calvenzani, Katia Petroni, Chiara Tonelli

Department of Biomolecular Sciences and Biotechnologies, University of Milan, Via Celoria 26, 20133 Milan (Italy)

In a statistical analysis of over 500 eukaryotic promoters, including many from plant species, the CCAAT box was one of the most ubiquitous elements, being present in 30% of them. In yeast and mammals, the CCAAT box is recognized by NF-Y, a trimer composed of distinct subunits: NF-YA, NF-YB and NF-YC, all required for DNA-binding. The NF-YB and NF-YC subunits form a tight dimer, via protein structures similar to the Histone Fold Motif -HFM- a conserved protein-protein and DNA-binding interaction module. Heterodimerization results in a surface for NF-YA association and the trimer can then bind to DNA with high specificity and affinity. In *Arabidopsis thaliana*, the complete NF-Y family is composed of 29 genes: 10 NF-YAs, 10 NF-YBs and 9 NF-YCs. We studied two NF-Y genes of the *AtNF-YA* sub-family, which are very close in the phylogenetic tree and both expressed in vegetative and reproductive tissues. Our aim was the characterization of their functional role.

Detailed expression analysis revealed that they have a similar expression pattern: they show low expression during ovule and pollen development and high expression during embryo development. Single mutants do not show any phenotypic defect and in order to verify the functional relationship between these genes, we decided to analyze the double mutants. These are lethal and the segregation analysis of plants homozygous for one gene and heterozygous for the other gene revealed that there is embryonic lethality. Morphological characterization of the progeny of these plants confirmed the presence of embryos arrested at the globular stage and characterized by irregular shape. These data indicate that these *AtNF-YA* genes are redundant and that they have a role at the globular stage of embryo development. Further analysis are underway to determine their specific function in embryogenesis.

P109 Analysis of RBR1 protein level and phosphorylation status in auxin-treated Arabidopsis roots

Pál Miskolczi1,2, Krisztina Ötvös1,2, Errin Johnson3, Zoltán Magyar2,4, László Bakó1,2

1Umea Plant Science Center, Umea University, Department of Plant Physiology, Umea, Sweden, 2Institute of Plant Biology, Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary, 3Umea Plant Science Center, Department of Forest Genetics and Plant Physiology, SLU, Umea, Sweden, 4Royal Holloway, University of London, School of Biological Sciences, Egham, UK

Unlike most plant organs, lateral roots do not originate from meristems but from an already differentiated tissue, the pericycle. Pericycle cells that will form a new lateral root first dedifferentiate and regain cell division activity then later differentiate into distinct cell types. The plant hormone auxin plays a key role in this process. Pericycle cells are believed to be in the G1 phase of the cell cycle until local auxin accumulation triggers them to proceed with the G1-S transition and start the cell division cycle, thereby initiating growth of a new lateral root. Treatment of Arabidopsis seedlings with the synthetic auxin 1-naphtylacetic acid (NAA) results in nearly synchronous initiation and growth of lateral roots. Conversely, while another synthetic auxin 2,4-Dichlorophenoxyacetic acid (2,4-D) triggers cell division and primordium formation, further lateral root development is inhibited. To investigate the underlying molecular mechanism by which these auxins act, we studied the auxin response of a cell cycle regulator, the Retinoblastoma-like protein 1 (RBR1). We found that the abundance, phosphorylation status and association properties of RBR1 in Arabidopsis roots are differentially affected by NAA and 2,4-D. This links auxin accumulation with RBR1 activity during lateral root formation in Arabidopsis.

P110 *Cis* element and transcriptome based screening of novel root hair-specific genes and their functional characterization in Arabidopsis

Su-Kyung Won, Yong-Ju Lee, Yoon-Kyung Heo, Misuk Cho, Hyung-Taeg Cho

School of Biological Sciences, Seoul National University, Seoul 151-742, Korea

Understanding the cellular differentiation of multicellular organisms requires the characterization of genes whose expression is modulated in a cell type–specific manner. The *Arabidopsis thaliana* root hair cell is one model for studying cellular differentiation. In the present study, novel

root hair cell specific genes were screened by a series of *in silico* and experimental filtration procedures. This process included genome-wide screening for genes with a well-defined root hair specific cis-element (RHE) in their promoter; filtering root-specific genes from the RHE-containing genes; further filtering of genes that were suppressed in root hair defective plant lines; and experimental confirmation by promoter assay. These procedures yielded many unstudied root hair-specific genes, including many protein kinases and cell wall related genes. Functional analysis of these root hair genes with loss-of-function mutants and over-expressing transformants revealed that they play roles in hair growth and morphogenesis. This study demonstrates that a defined cis-element can serve as a useful starting point for the genome-wide screening of cell type specific genes, and implicates novel root hair specific genes in root hair development.

This study was supported by grants from KOSEF, CFGC, BioGreen 21, and EBNCRC.

P111 Resetting and regulation of *Flowering Locus C* expression during Arabidopsis reproductive development

Jean Choi1, Youbong Hyun1, Min-Jeong Kang1,2, Hye In Yun1, Jae-Young Yun3, Clare Lister4, Caroline Dean4, Richard M Amasino2,3, Bosl Noh2,5, Yoo-Sun Noh1,2, Yeonhee Choi1

1Department of Biological Sciences, Seoul National University, Seoul, 151-742, Korea., 2Global Research Laboratory for Floral Regulatory Signaling at SNU & UW, Seoul National University, Seoul 151-742, Korea., 3Department of Biochemistry, University of Wisconsin, Madison, Wisconsin 53706, USA., 4Department of Cell and Developmental Biology, John Innes Centre, Norwich, NR4 7UH, UK., 5Environmental Biotechnology National Core Research Center, Gyeongsang National University, Jinju, 660-701, Korea.

The epigenetic regulation of the floral repressor *FLOWERING LOCUS C (FLC)* is one of the critical factors that determine flowering time in *Arabidopsis thaliana*. Although many *FLC* regulators and their effects on *FLC* chromatin have been extensively studied, the epigenetic resetting of *FLC* has not been thoroughly characterized. Here, we investigate the *FLC* expression during gametogenesis and embryogenesis using *FLC::GUS* transgenic plants and RNA analysis. Regardless of epigenetic state in adult plants, *FLC* expression disappeared in gametophytes. Then, *FLC* expression was reactivated after fertilization in embryos but not in the endosperm. The *FLC* expression in embryos was equally contributed by both parental alleles. Surprisingly, reactivation of *FLC* in early embryos was independent of FRIGIDA (FRI) and *SUPPRESSOR OF FRIGIDA 4 (SUF4)* activities. Instead, FRI, *SUF4*, and autonomous-pathway genes determined the level of *FLC* expression only in late embryogenesis. Many *FLC* regulators exhibited expression patterns similar to that of *FLC*, indicating potential roles in *FLC* reprogramming. An *FVE* mutation caused ectopic expression of *FLC* in the endosperm. A mutation in *PHOTOPERIOD-INDEPENDENT EARLY FLOWERING 1* caused defects in *FLC* reactivation in early embryogenesis and maintenance of full *FLC* expression in late embryogenesis. We also show that polycomb-group complex components Fertilization-Independent Endosperm and MEDEA which mediate epigenetic regulation in seeds are not relevant for *FLC* reprogramming.

P112 Finding new developmental genes – a reverse genetics approach on Polycomb group protein (PcG) target genes

Julia Engelhorn1, Julia Reimer1, Sara Farrona1, Ulrike Göbel2, George Coupland1, Franziska Turck1

1Department of Plant Developmental Biology, Max Planck Institute for Plant Breeding Research, Cologne, Germany, 2Bioinformatics Support, Max Planck Institute for Plant Breeding Research, Cologne, Germany

We pursue a reverse genetics approach to identify new genes involved in plant development by utilizing the genome wide distribution data of the Polycomb group protein (PcG) target genes. Gene repression via the Polycomb repressive system ensures normal development in plants and animals. In animals, several Polycomb Repressive Complexes (PRC1-PRC4) are present, whereas in plants only PCR2 is conserved. PRC2 catalyzes trimethylation of lysine 27 of Histone 3 (H3K27me3) in plants and animals, PCR1 recognizes this mark in animals. In Arabidopsis TERMINAL FLOWER 2/LIKE HETEROCHROMATIN PROTEIN 1 (TFL2/LHP1) can bind to H3K27me3 *in vitro* and co-localizes with H3K27me3 *in vivo*. Loss of TFL2/LHP1- function leads to defects in timing of flowering and plant anatomy, partial loss of H3K27me3 leads to formation of a callus like structure, emphasizing the importance of PcGs and their targets in plant development. To select candidate genes among PcG targets we performed a transcriptional cluster analysis to obtain genes expressed in the shoot apex where major developmental processes take place. A Gene Ontology (GO) analysis of these genes revealed an overrepresentation of developmental functions. Since 50% of the genes in this cluster are not characterized in detail yet, we hypothesize that they might also play a role in development. To test this hypothesis we are currently characterizing T-DNA insertion lines for these genes in long and short day growth conditions. So far we could identify one stably late flowering insertion line as well as one homozygotic lethal knock out of a candidate gene. Five other abnormal phenotypes were observed in a first screening but need to be verified.

P113 SAUL1, a novel E3 ubiquitin ligase, is required for suppression of premature senescence and cell death

Gabriele Drechsel1, Sabine Raab1, Florian Bittner2, Stefan Hoth1

1Molecular plant physiology, FAU Erlangen, Germany, 2Department of Plant Biology, Technical University of Braunschweig, Germany

Plant senescence is a developmentally and genetically well-defined process. Components of the regulatory network controlling leaf senescence in Arabidopsis have been identified by genetic screens for senescence mutants and by gene expression screens for senescence-

associated genes. However, the underlying molecular mechanisms of senescence regulation are not yet fully understood.

Here we present data showing a role of a novel E3 ubiquitin ligase, SAUL1 (Senescence Associated E3 Ubiquitin Ligase1, also named PUB44), in suppression of premature senescence and cell death. Plants defective in the *SAUL1* gene grow normally under high photon flux densities (PFD). However, *saul1* plants show a premature senescence phenotype when transferred to low PFD at any developmental age, but also when exposed to other stress conditions under permissive light. Therefore *saul1* plants represent an ideal inducible model system to study the onset and progress of senescence.

We show that in *saul1* mutants senescence symptoms are accompanied by an increase in ABA biosynthesis. The rise in ABA levels is caused by enhanced activity and accumulation of Aldehyde Oxidase 3 (AAO3), the enzyme catalyzing the last step of ABA biosynthesis. In label transfer experiments, we were able to show transient interactions between SAUL1 and AAO3 proteins. Therefore we suggest that SAUL1 participates in targeting AAO3 protein for ubiquitin-dependent degradation via the 26S proteasome pathway in order to prevent premature senescence. In addition to AAO3, several other putative interaction partners of SAUL1 identified by a yeast-two-hybrid screening will be presented.

P114 EPF2 regulates stomatal development

Lee Hunt, Julie Gray

Molecular Biology & Biotechnology Department, University of Sheffield, Sheffield, UK

Stomata are pores in the plant epidermis that control carbon dioxide uptake and water loss. They are major regulators of global carbon and water cycles. Several signaling components have been characterised which regulate stomatal development in Arabidopsis. These include a putative secretory peptide EPF1, LRR receptor components TMM and ER, and a peptidase SDD1. Loss of function in these genes alters the number of cells that enter the stomatal lineage and how they are spaced in relation to each other on the leaf surface. We have identified EPF2, a peptide related to EPF1 that is expressed in proliferating cells of the stomatal lineage known as meristemoids, and in guard mother cells, the progenitors of stomata. In the absence of EPF2 excessive numbers of cells enter the stomatal lineage and produce numerous small epidermal cells that express stomatal lineage reporter genes, whereas plants over-expressing EPF2 produce virtually no stomata. Results from genetic experiments indicate that EPF2 regulates a different aspect of stomatal development to EPF1, and are consistent with EPF2 acting in a pathway to regulate stomatal density that involves ER and TMM, but not SDD1. We propose that EPF2 is involved in determining the number of cells that enter, and remain in, the stomatal lineage. Here we also discuss how other known, and unknown, factors may interact with EPF2 to influence stomatal patterning and how genetic interactors with EPF2 may be organ dependant.

P115 SQUINT is required for proper CLAVATA signalling

Nathanaël Prunet, Patrice Morel, Priscilla Champelovier, Anne-Marie Thierry, Ioan Negrutiu, Christophe Trehin

Laboratoire de Reproduction et Developpement des Plantes, Ecole Normale Superieure de Lyon, France

Mutation of *SQUINT (SQN)*, which encodes the Arabidopsis ortholog of cyclophilin 40, causes various phenotypes, including a precocious transition to the adult phase, altered phyllotaxy and an increase in carpel number (Berardini *et al*, 2001). *SQN* also controls flower meristem (FM) termination redundantly with other factors, a function that appears to be mediated by transcriptional regulation of *AGAMOUS* in the very centre of the flower bud (Prunet *et al*, 2008).

A closer examination revealed that *sqn* loss-of-function mutants also exhibit enlarged shoot apical meristem (SAM) defects. This phenotype, together with the altered phyllotaxy, increased carpel number and delayed FM termination, makes *sqn* very similar to weak *clavata (clv)* mutants and prompted us to investigate the relations between *SQN* and the *CLV* pathway. Contrary to other mutations causing similar phenotypes, *sqn* has a very mild effect in a *clv* mutant background, suggesting that *SQN* and the *CLV* genes act in the same genetic pathway. Accordingly, loss-of-function of *SQN* nearly fully rescues the meristem abortion phenotype of plants overexpressing *CLV3*, showing that it is required for constitutive CLV signalling.

Berardini TZ, Bollman K, Sun H, Poethig RS (2001) Regulation of vegetative phase change in *Arabidopsis thaliana* by cyclophilin 40. Science 291: 2405-2407

Prunet N, Morel P, Thierry AM, Eshed Y, Bowman JL, Negrutiu I, Trehin C (2008) REBELOTE, SQUINT, and ULTRAPETALA1 Function Redundantly in the Temporal Regulation of Floral Meristem Termination in *Arabidopsis thaliana*. Plant Cell

P116 The role of a novel transcriptional complex in maintaining leaf polarity and meristem activity in Arabidopsis

Melissa Stahle1, Janine Kuehlich1, Lindsay Staron2, Albrecht von Arnim2, John Golz1

1Department of Genetics, University of Melbourne, Melbourne, Australia, 2Department of BCMB, University of Tennessee, Knoxville, TN 37996, USA

In Arabidopsis, patterning along the adaxial-abaxial axis of the leaf requires the activity of several families of transcription factors that include the abaxially expressed *YABBY* genes, *FILAMENTOUS FLOWER (FIL) and YABBY3 (YAB3)*. Given that two other *YABs (YAB2, YAB5)* are expressed vegetatively, we investigated whether these *YABs* also share redundant functions with *FIL* and *YAB3*. Unlike *yab2, yab5* mutants enhanced the *fil yab3* leaf polarity defect. Surprisingly triple mutant leaves are severely abaxialised, revealing that *YABs* primarily promote adaxial cell identity.

To investigate *YABs* function further, *FIL* was fused to either VP16 or an EAR motif and introduced into *fil yab3* mutants under the control of its native promoter. While the

FIL-VP16 fusion partially complemented the *fil yab3* leaf phenotype, *fil yab3* plants expressing the FIL-EAR transgene were phenotypically similar to *fil yab3 yab5* plants. A possible explanation for this phenotype is that YABs function as heterodimers and that dimers incorporating FIL-EAR are no longer functional. According to this model FIL-EAR inactivates YAB5 in a *fil yab3* mutant background resulting in a *yab* triple mutant phenotype. We present extensive yeast two hybrid and bioluminescence resonance energy transfer (BRET) data that show that YABs are capable of forming both homo and heterodimers.

We also present data showing that YABs physically and genetically interact with two closely related transcriptional co-repressors LEUNIG (LUG) and LEUNIG-HOMOLOG (LUH). In addition to regulating organ polarity, KNOX gene expression and flower development, we show that the YAB-LUG complex promotes SAM formation and maintenance. Using artificial miRNAs targeted to *LUG* and *LUH* mRNA, we demonstrate that the SAM promoting pathway regulated by these co-repressors is likely to be active in leaves. As *YAB* expression is confined to the abaxial domain of developing organs, we propose that the YAB-LUG complex regulates a signaling pathway required for SAM formation and maintenance.

P117 *In vivo* targets of FPA, an RNA binding protein controlling Arabidopsis flower development

Lionel C Terzi, Gordon G Simpson

University of Dundee, Dundee, UK

The switch to floral development is quantitatively controlled by the integration of multiple pathways responding to environmental cues (such as ambient temperature and daylength) and an endogenous program of development. RNA processing factors feature prominently in the control of flowering time and the pre-mRNAs of most genes involved in flowering time control are alternatively processed (Terzi LC and Simpson GG, 2008, Curr Top Microbiol Immunol. 2008;326:201-18). *FPA* encodes an RNA-binding protein comprising three RNA Recognition Motifs (RRM) and a SPOC domain. Loss-of-function *fpa* mutants flower late, revealing that *FPA* normally functions to promote flowering. In addition, *FPA* has functions outside flowering time control, such as the regulation of targets of the RNA-mediated DNA methylation pathway. We will present our tiling microarray analysis of RNA purified from *fpa* mutants, revealing previously unidentified genetic targets of *FPA*. To address the directness by which FPA controls expression of its targets, we have developed an RNA immunoprecipitation (RIP) protocol for use with Arabidopsis (Terzi LC and Simpson GG, 2009, The Plant Journal, Published Online Mar 27 2009). Working with the well established interaction of U2B with U2 snRNA as a system with which to optimise the procedure, we routinely enrich U2 over other RNAs eg U1, in a manner dependent on *in vivo* cross-linking. Immunoprecipitation of U2B fused to GFP was performed using commercially available anti-GFP antibodies, enabling our RIP protocol to be applied straightforwardly to any other Arabidopsis RNA-binding proteins. Using antibodies to FPA and loss-of-function *fpa* mutant background as a negative control, we are currently performing RIP analysis with FPA in combination with tiling array identification of associated RNA. Therefore, with this preliminary study, we are directly linking RNA processing events controlling development to the proteins that regulate them. In the future, the combination of Solexa sequencing of Arabidopsis mutants defective in RNA binding proteins with the RIP procedure offers huge potential to reveal how Arabidopsis RNA binding proteins function.

P118 The role of P5CS1 and P5CS2 in the flower transition of *Arabidopsis thaliana*

Roberto Mattioli1, Stefania Nigro1, Franco Abbate1, Giuseppina Falasca2, Maria Maddalena Altamura2, Paolo Costantino1, Maurizio Trovato1

1Dipartimento di Genetica e Biologia Molecolare, Universite di Roma La Sapienza, Rome, Italy, 2Dipartimento di Biologia Vegetale, Universite di Roma La Sapienza, Rome, Italy

Proline synthesis proceeds from glutamate in a two-step reaction controlled by the enzyme Δ1-pyrroline-5 carboxylate synthetase (P5CS), which in Arabidopsis is encoded for by the two paralog genes *P5CS1* and *P5CS2*. We recently shown that *P5CS1* overexpression results in proline accumulation and precocious flowering in early stages of Arabidopsis development, while mutations in *P5CS1* results in proline reduction and late flowering, implying a role for proline in flower transition. The *p5cs1* mutant, however, exhibits only a modest delay in flowering, indicating that *P5CS1* and *P5CS2* play overlapping roles in flower transition. Accordingly, we are currently analyzing the expression of either *P5CS1* or *P5CS2*, by *in situ* hybridization and qRT-PCR. In particular, we are focusing our attention on the reproductive phase of Arabidopsis and will present data on the expression of *P5CS1* and *P5CS2* in the vegetative and floral shoot apical meristem, in the axillary buds, and in the siliques. Furthermore, since *p5cs2* mutants are embryo lethal, as recently reported and experimentally confirmed in this communication, we crossed homozygous *p5cs1/p5cs1* with heterozygous *p5cs2*/+ mutants to evaluate the possible contribution of *P5CS2* in flowering time. Data on the flowering time of *p5cs1/p5cs1* and *p5cs2/+* will be presented and discussed. Further work is in progress to define better the role of *P5CS1* and *P5CS2* in flower transition and to understand which floral pathway proline is involved in.

P119 DETORQUEO, QUIRKY, and ZERZAUST, novel components involved in organ development mediated by the receptor-like kinase STRUBBELIG

Lynette Fulton1, Martine Batoux1, Prasad Vaddepalli1, Ram Kishor Yadav1, Wolfgang Busch2, Stig Anderson2, Sangho Jeong 3, Jan Lohmann 2,4, Kay Schneitz1

1Entwicklungsbiologie der Pflanzen, Wissenschaftszentrum Weihenstephan, Technische Universitet Munchen, Freising, Germany, 2Max Planck Institute for Developmental Biology, Department of Molecular Biology, AG Lohmann, Tubingen, Germany, 3Section of Cell and Developmental Biology, University of California San Diego, La Jolla, California, United States of America, 4Center for Organismal Studies, University of Heidelberg, Heidelberg, Germany

Plant organs, such as leaves and flowers, arise through cellular division events that are precisely coordinated

between both adjacent, related cells and across distinct cell layers. Receptor-like kinases are cell surface receptors that perceive and relay intercellular information. In Arabidopsis, the receptor-like kinase STRUBBELIG has been shown to mediate inter-cell layer communication during floral development, amongst other functions; little is known, however, concerning its exact signaling mechanism. In order to identify additional molecular components involved in SUB-dependent signaling processes, we used forward genetics and identified recessive mutations in three genes that result in a sub-like phenotype. Plants with a defect in *DETORQUEO (DOQ), QUIRKY (QKY),* and *ZERZAUST (ZET)* show corresponding defects in outer integument development, floral organ shape and stem twisting, and also show sub-like cellular defects in the floral meristem and roots. Thus, *SUB, DOQ, QKY,* and *ZET* define the *STRUBBELIG-LIKE MUTANT (SLM)* class of genes. Morphological analysis of single and double mutants indicated that SLM genes have overlapping, but also distinct, functions in plant organogenesis. Systematic comparison of whole-genome transcript profiles defined common and distinct sets of transcriptional targets in slm mutants, thus supporting this notion. Here we describe the molecular nature of *QUIRKY*: the encoded protein is likely membrane-localized and predicted to require Ca^{2+} for activity. Our current hypothesis is that QUIRKY facilitates transport of molecules to the cell boundary and may support a STRUBBELIG-related extracellular signal.

P120 Novel regulators and interactions controlling plant architecture and development

Enriqueta Alos, Philip A Wigge

Cell and Developmental Biology Department, John Innes Centre, Colney, NR4 7UH, Norwich, UK

While the key components of the floral transition have been discovered in Arabidopsis, less is known of the mechanisms by which information of the floral transition is used to coordinate other aspects of development, especially plant architecture.

In Arabidopsis, a key role in maintaining indeterminate vegetative meristems is carried out by *TERMINAL FLOWER 1 (TFL1)*, a gene closely related to the floral pathway integrating gene *FLOWERING LOCUS T (FT)*. While *ft* mutants are very late flowering, *tfl1* mutations have the opposite phenotype, causing the plants to flower early and terminate their growth with a profusion of flowers, resembling gain-of-function *35S::FT* plants.[1,2,3]

To address both plant architecture and the limiting role of *FT* in plant development, we have performed a genetic screen for flowering time and architecture mutants in the *tfl1-1* background. 3400 M2 families have been screened and 20 mutants selected as modifiers of the *tfl1-1* phenotype. Amongst the isolated mutants, mutations in already described genes have been identified such as *APETALA 1* and *LEAFY*. Interestingly, novel modifiers of the *tfl1-1* phenotype have been obtained and mapped. The functional characterization and the roles of these genes in plant architecture will be described.

1 Bradley, D., Ratcliffe, O., Vincent, C., Carpenter, R., and Coen, E. (1997). Science 275, 80-83.
2 Kardailsky, I., Shukla, V.K., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T., Chory, J., Harrison, M.J., and Weigel, D. (1999). Science 286, 1962-1965.
3 Prusinkiewicz, P., Erasmus, Y., Lane, B., Harder, L.D., and Coen, E. (2007). Science 316, 1452-1456.

P121 Dissecting the transcriptional cascade in auxin-induced cell fate re-specification

Shuai Yuan, Bastiaan Bargmann, Puileng Ip, Kenneth Birnbaum

Center for Genomics and Systems Biology, New York University

How cells adopt specific cell fates during organogenesis remains a fundamental question in developmental biology. Arabidopsis roots cells can respond to positional information and take on different cell fates. However, the molecular nature of positional information remains to be identified and how the positional information is perceived by cells and builds specific cell types is poorly understood. It has been found that the plant hormone auxin can induce ectopic quiescent center (QC) and columella identities in specific cell types. We revisited this experiment and found that a QC-specific marker can be induced in the endodermis and cortex upon auxin treatment, and that a columella-specific marker can be induced in the epidermis. This provides us a very nice system to re-specify cell fate *in vivo* by simply treating the plants with auxin, which allows us to assess how auxin causes this identity change. We hypothesize that auxin triggers a transcriptional cascade that builds QC or columella cell fates in endodermis/cortex and columella, respectively. We aim to test this hypothesis by finding the components of the QC/columella-specific transcriptional cascades responding to auxin treatment. While the cells are changing their identity on auxin, we can use fluorescence activating cell sorting (FACS) to sort out these re-differentiating cells by taking advantage of the stability of the GFP markers specifically expressed in these cells. Then we can examine their cell-type-specific transcriptional profiles by microarray experiments using RNA from the cells that are changing their identity. If we sample along the cell identity transition stage, we should be able to find the genes turned on upon auxin signaling and put them in order. These genes represent at least part of the transcriptional cascade that builds the quiescent center or columella cells.

P122 Identification of genes implicated in lateral root development in *Arabidopsis thaliana*

Concepcion Manzano1, Bert De Rybel2, Tom Beeckman2, Carlos Del Pozo1

1Centro de Biotecnologia y genomica de plantas (CBGP), 2Department of Plant Systems Biology, VIB, Technologiepark 927

Plant root system development can be adapted to many environmental conditions. Lateral root initiation is an essential and continuous process during the formation of root systems, which mainly depends on the number and position of the lateral roots (LR) along the main root axis. In comparison to the formation of the aerial ramifications,

which develop from auxiliary meristems, roots lack auxiliary meristems. Lateral roots are formed from the founder cells, which are located in the pericycle layer. These founder cells are arrested at the G1 phase of the cell cycle and in response to auxin, they are capable of re-activating cell division to form lateral root primordial. However, the molecular and genetic components that determine the position of lateral roots are still unknown.

We have identified a gene, that is expressed in the LRIP (Lateral Root Initiation Point) and we call it *LRIP1*. This gene is expressed in dividing areas (root and shoot meristems), and more interestingly, along the main root in discrete patches, and some of them correspond to founder cells that have not divided yet. This expression pattern has been denominated 'lateral root initiation point' (*LRIP*). We have developed a transgenic plant that harbours *LRIP* promoter region fused to GFP reporter gene. To identify molecular components that control the pericycle cell division to form lateral root we carried out a transcriptomic analysis of root fluorescent-activated cell sorting using LRIP1::GFP root protoplasts. We have identified 200 genes that are significantly expressed in these founder cells. The reproducibility of the expression profiles, the resolution of our experimental approach, and the potential involvement in lateral root development will be evaluated with overexpressors and mutants of some of these identified genes.

P123 Towards the identification of egg cell regulators

Ronny Völz, Rita Gross-Hardt

ZMBP Developmental Genetics, University of Tubingen, Germany

In plants, egg and sperm cells are formed within few celled haploid gametophytes. The key reproductive cells of the female gametophyte, egg and central cell, get fertilized to form the main seed components. These gametic cells are flanked by accessory cells that aid in fertilization. In a screen for regulators of egg cell identity we identified the *lachesis (lis)* mutant. In *lis* gametophytes all cells differentiate either egg or central cell fate, indicating that all female gametophytic cells are competent to adopt gametic cell fate. A *LIS* promoter reporter construct confers expression to egg and central cells of mature female gametophytes. In order to identify regulators of egg cell fate we employed the *LIS* promoter as a tool and carried out promoter deletion studies. We identified a 72bp cis-regulatory element necessary for egg cell expression of *LIS*. On the basis of this fragment we performed a yeast one-hybrid assay and isolated 12 transcription factors as putative binding components. A preliminary characterization of the candidate genes will be presented.

P124 The role of NF-Y transcription factors in flowering

Roderick Kumimoto, Yan Zhang, Nicholas Siefers, Ben Holt III

University of Oklahoma, Norman, Oklahoma, USA

The heterotrimeric NUCLEAR FACTOR Y (NF-Y) transcription factor families have undergone extensive duplication in the plant lineage. While metazoans tend to have only one copy of each subunit gene, Arabidopsis, as well as other examined angiosperms, have greater than 10 *NF-Y* genes in the three distinct families (10 *NF-YA*, 13 *NF-YB*, and 13 *NF-YC*). Individual subunits have been shown to have functions in drought tolerance, maintenance of nitrogen-fixing nodule meristems, embryo development, and flowering time. Currently, there is no plant process for which the complete NF-Y complex has been described (from animal systems, there is an expectation that functional NF-Y transcription factors will include one subunit from each family). To help identify complete NF-Y complexes, we recently developed stable promoter:GUS fusions for all 36 Arabidopsis NF-Y (Siefers *et al*, 2008. PPhys 149:625-641). We are currently utilizing these lines to identify complete, floral promoting NF-Y complexes. It is already known that NF-YB2 and NF-YB3 have over-lapping function in the photoperiod dependent flowering pathway, we are focusing our efforts on the NF- YC and NF-YA components. Using the promoter:GUS lines, we have identified three *NF-YC* genes that are 1) simultaneously expressed in the leaf vasculature with *NF-YB2, NF-YB3, CONSTANS (CO)*, and *FLOWERING LOCUS T (FT)*, and 2) have over-lapping function required for photoperiod dependent flowering, and 3) are genetically required for the promotion of early flowering by constitutively expressed CO. Further, these NF-YC interact *in vivo* with NF-YB2 and NF-YB3.

We currently estimate that at least 12 unique NF-Y complexes are involved in flowering. At the ICAR meeting, we will discuss these results and progress towards further characterizing the functions of NF-YA in flowering, as well as current theories on the roles of NF-Y in the plant lineage.

P125 Kiss of Death (KOD) is a novel peptide regulator of Programmed Cell Death in Arabidopsis

Bennett Young, Robert Blanvillain, Patrick Gallois

University of Manchester, The Faculty of Life Sciences, Michael Smith Building, Oxford Road, Manchester, M13 9PT, UK

Programmed cell death (PCD) is an evolutionarily conserved process essential to all multicellular organisms. Plants are no different in this case and utilise PCD in a wide range of situations particularly in their development (xylogenesis and embryogenesis) and defence (hypersensitive response to pathogens). Here we describe a novel 25-amino-acid peptide named KOD, whose expression was first detected in the terminally differentiated cells of the suspensor, an organ known to undergo PCD in embryos. Upon further analysis, *KOD* transient overexpression was found to induce death in Arabidopsis protoplasts, tobacco leaves, and onion cells. Furthermore, two *KOD* mutant lines showed PCD phenotypes enforcing *KODs* role as a possible positive regulator of PCD, including in the progression of cell death of the suspensor. Finally, we used a transient expression system to measure cell survival using biolistics and fluorescent techniques in onion epidermal cells. The expression of mutant variants of *KOD* in this system has demonstrated the importance of particular residues in KODs sequence, which are essential to its function. In addition, we used

this system to show that *KOD* acted upstream of the caspase-like activities detected during Arabidopsis PCD. In conclusion, we are presenting data on the analysis of *KOD*, a novel gene, which we propose to be a novel positive regulator of PCD in Arabidopsis.

P126 SIAMESE cooperates with a CDH1-like protein to establish endoreplication in Arabidopsis trichomes

John Larkin, Remmy Kasili, Jason Walker, Alice Simmons, Jing Zhao

Louisiana State University, Baton Rouge, LA, USA

During the development of multicellular organisms, cell differentiation is tightly coordinated with cell division. In some cell types, a modified cell cycle occurs during differentiation in which the DNA is replicated without concomitant cell division, resulting in an increase in nuclear DNA content. This process is called endoreplication; in plants, important examples of endoreplicated cell types include cereal endosperm, cotton fibers, and nitrogen-fixing symbiotic nodules in legumes. The coordination of this modified cell cycle with cell differentiation remains poorly understood.

The *SIAMESE (SIM)* gene is a key regulator of endoreplication during Arabidopsis trichome (leaf hair) development. The SIM protein is the defining member of a plant-specific family of cyclin-dependant kinase (CDK) inhibitors that bind to D-cyclin-containing CDK complexes. We have identified a gene, *ENHANCER OF SIM2 (ENS2)*, as a genetic modifier of the multicellular trichome phenotype of *sim-1* mutants. Loss-of-function *ens2* mutations dramatically enhance the multicellularity of sim mutants trichomes in double mutants, and over-expression of *ENS* completely suppresses the sim mutant phenotype. *ENS2* encodes the CDH1-like protein CCS52A1. These results, as well as interactions between *ens2* and constructs overexpressing various cell cycle regulators, suggest that *ENS2* cooperates with *SIM* in establishing the endocycle, and shed light on the relative roles of a CDK inhibitor and a CDH1-like protein in controlling endoreplication in plants.

P127 Functional domains of PETAL LOSS, a trihelix transcription factor in *Arabidopsis thaliana*

Tezz L Quon, David R Smyth

Monash University, Clayton, Victoria, Australia

Floral architecture is controlled by a symphony of different genes. The *PETAL LOSS* (*PTL*, At5G03680) gene is expressed in the region between developing sepals, adjacent to the region where petals arise. Its function is to apparently suppress growth in these regions. *PTL* is a member of the plant specific trihelix family of transcription factors. The PTL protein contains two putative MYB-like DNA binding domains, known as trihelix domains. Located between these is a central conserved domain, likely involved in protein-protein interactions. We have previously shown that, as with other members of this family, PTL can homodimerize.

Key residues involved in DNA binding have been identified by site directed mutagenesis and its ability to affect ptl complementation. We provide evidence that the N-terminal trihelix is essential for PTL function, and that the C-terminal trihelix is not essential but also plays a role. By replacing the trihelix domains of *PTL* with the corresponding domains of closely related genes, such as GT-2 (At1G76890) and *SISTER OF PETAL LOSS* (*SOP*, At3G10000), we have evidence that the targets of PTL are not shared with GT-2 but are shared with SOP. Bimolecular Fluorescence Complementation (BiFC) results have shown that PTL is capable of forming heterodimers with related trihelix proteins such as GT-2 and DF-1 (At1G76880). BiFC, combined with site directed mutagenesis was used to identify multiple amino acids within the central conserved domain that are involved in these protein-protein interactions.

We have shown that the duplicate trihelix DNA binding domains of PTL are differentially important for its function and that the central conserved domain is involved in protein-protein interactions. Further work is required to elucidate the exact role of these domains in helping establish floral architecture.

P128 The role of INSENSITIVE TO LOW TEMPERATURE 3 (ILT3) in the control of flowering time by ambient temperature in Arabidopsis

Jeong Hwan Lee, Kyung Eun Kim, Richard G H Immink, Jong Seob Lee, Ji Hoon Ahn

Seoul National University, Seoul

Plants show remarkable developmental plasticity to survive in a continually changing environment such as photoperiod, light quality, vernalization, and ambient growth temperature. In contrast to light and vernalization, little is known about a genetic pathway (thermosensory pathway) that mediates the responses to the change of ambient temperature. Here, we identified two ecotypes that respond only weakly to a change in ambient temperature by natural variation approach and found a deletion of genetic loci that affects weak temperature insensitivity. Furthermore, we confirmed weak temperature response in T-DNA alleles of the loci at 23°C or 16°C, and renamed it ILT3 (INSENSITIVE TO LOW TEMPERATURE 3). The early flowering of ilt3 mutants at 23°C or 16°C under long day (LD) conditions resulted from upregulation of *FLOWERING LOCUS T (FT)* and *TWIN SISTER OF FT (TSF)*, suggesting that ILT3 is a floral repressor. The genetic interaction of *ilt3* with other floral repressor mutants (*svp* and *flc* mutants) showed that the flowering time of svp and flc mutants was accelerated by the *ilt3* mutation, and the temperature insensitivity was also maintained in *svp ilt3* and *flc ilt3* double mutants. However, the expressions of *SHORT VEGETATIVE PHASE (SVP)* and *FLOWERING LOCUS C (FLC)* were unchanged in absence of ILT3, and ILT3 expression remained unaffected by increases or reductions in SVP and FLC activities. This result indicates that ILT3 acts independently of SVP and FLC at the transcriptional level. Insensitivity of temperature response of *ilt3* was suppressed by loss of ft and tsf function,

whereas gain of FT function was epistatic to *ilt3*. Interestingly, ILT3 interacts with SVP *in vivo* and *in vitro*. These data suggest that ILT3 is another component within the thermosensory pathway and the interaction between ILT3 and SVP mediated the effects of ambient temperature.

P129 A disease resistance regulator, MAP kinase 4, is required for male-specific cytokinesis in Arabidopsis

QingNing Zeng1, Brian E Ellis2, Jin-Gui Chen1

1Department of Botany, University of British Columbia, Vancouver, BC V6T 1Z4, 2Michael Smith Laboratories, University of British Columbia, Vancouver, BC V6T 1Z4

Plant reproduction is crucial not only for the existence of the species itself, but also for the world's food supply. Successful production of progeny relies on the formation of viable male and female gametes. Here we report that AtMPK4, a mitogen-activated protein kinase that was previously described as a regulator of disease resistance, also specifically regulates post-meiotic male-specific cytokinesis in Arabidopsis. Although *mpk4* mutants form smaller but complete flowers, the anthers contain few, but highly enlarged pollen grains, which exhibit, reduced viability. DAPI staining demonstrated that the mature *mpk4* pollen grains have increased sets of the tricellular structure, which is composed of two germ cells and one vegetative cell. Closer examination of the pollen formation process revealed that *mpk4* cannot undergo proper cytokinesis after the completion of male meiosis, and that the callose walls that normally surround the tetrads in wildtype plants are absent in *mpk4*. Promoter-reporter analysis showed that *AtMPK4* is expressed in anthers, consistent with its function in male gametophyte formation.

Since yeast two-hybrid assays demonstrated that AtMPK4 interacts strongly with AtMKK6, a MAP kinase kinase that was previously shown to be involved in cytokinesis, we propose that AtMKK6 and AtMPK4 form a MAP kinase signalling module that specifically regulates male meiotic cytokinesis in Arabidopsis.

P130 Arabidopsis DNA contains very few putative G-quadruplex sequence motifs

Ayami Nakagawa1, Hiro Takahashi1, Nobuo Sato1, Shoko Kojima1, Byung Yoon Cha2, Je-Tae Woo2, Yasunori Machida3, Chiyoko Machida1

1Plant Biology Research Center, Chubu University, Aichi, Japan, 2Research Institute for Biological Functions, Chubu University, Aichi, Japan, 3Graduate School of Science, Nagoya University, Aichi, Japan

Guanine-rich DNA sequences often form G-quartets, planar arrays of four guanines stabilized by monovalent cations (K^+ and Na^+); which interact to form a G-quadruplex. In telomeres, G-rich sequences (repeats of TTTAGGG) form a G-quadruplex that interferes in the elongation reaction by telomerase. Recent bioinformatics analysis revealed that G-quadruplex-forming sequences exist ubiquitously in mammalian and bacterial genomes, and are enriched in promoters and near the translation start sites. Ligand binding of a G-quadruplex may decrease transcription of downstream genes. These findings support the current hypothesis that G-quadruplex may be a novel type of ubiquitous regulatory element. Nonetheless, there are no studies on G-quadruplex sequences and its regulatory mechanism in plants. In this study we retrieved the G-quadruplex sequence in Arabidopsis. Sequence analysis showed that the Arabidopsis genome possesses approximately 1,200 G-quadruplex sequences. The frequency over the whole genome length is only one tenth of that in rice, fruit fly, or human. We also report on the changes of the levels of transcripts of genes nearest a G-quadruplex by the binding of its ligand.

P131 SD5, a homologue of spliceosome subunit, regulates proliferation in post-seedling development

Takeshi Yoshizumi1, Hiroaki Hongo1, Takashi Kuromori2, Yoko Horii1, Yuko Imura2, Asako Kamiya2, Hiroaki Shimada 3, Minami Matsui1

1Plant Functional Genomics Group, PSC, RIKEN, Japan, 2Gene Discovery Research Group, PSC, RIKEN, Japan, 3Department of Biological Science and Technology, Tokyo University of Science, Japan

Plant body size is thought to be tightly regulated by nuclear ploidy levels and cell numbers. To elucidate ploidy and cell cycle control, we analyzed *segregation distortion 5 (sd5)* originally identified as a marker non-mendelian inherited mutant from RIKEN *Ds* insertional mutant lines. *sd5* mutant consisted of small and abnormal shape leaves, and it showed various developmental arrest at post-seedling stage. Cell numbers were decreased and *CYCB1;1* expression was also down-regulated in *sd5* leaves, whereas *sd5* developed apparently normal shoot apical meristem. These indicate that the *sd5* mutation strongly affected cell proliferation rather than polyploidy.

sd5 encodes a homologue of yeast *DIM1*, a component of U5 spliceosome. Animal and plants possess two *DIM1* homologues, although single *DIM1* gene was found in yeast genome. Loss of *DIM1* caused lethality as a result of defect of cell cycle in *S. pombe.* Interestingly, *SD5* protein did not complement to the pombe *dim1-35* mutation when *SD5* was expressed under limited temperature. On the other hand Arabidopsis another homologue of *DIM1* could complement to this mutation, indicating this homologue acts as an authentic DIM1 in Arabidopsis (*AtDIM1*). Addition to yeast complementation, *AtDIM1* did not complement *sd5-2* mutation in Arabidopsis when the expression was driven by *SD5* promoter. These results suggested SD5 plays different functions of authentic DIM1 protein.

Here we will demonstrate the detail of *sd5* phenotypes and physiological functions of SD5.

P132 Functional characterization of BPEp bHLH transcription factor and its interacting protein, two transcription factors involved in the control of petal growth in *Arabidopsis thaliana*

Florian Brioudes1, Emilie Varaud1, Caroline Joly1, Judit Szécsi1,2, Julie Leroux1, Floriant Bellvert3, Cédric Bertrand 3, Mohammed Bendahmane1

1Reproduction et Developpement des Plantes UMR INRA-CNRS-Universite Lyon 1-ENSL, IFR128 BioSciences, Ecole Normale Superieure, 46 allee d'Italie 69364 Lyon cedex 07, France, 2EVIR, INSERM U758, ENS, 46 allee d'Italie 69364 Lyon cedex 07. France, 3Universite de Lyon, Lyon F-69003. Universite Lyon1, Villeurbanne F-69622. Centre d'Etude des Substances Naturelles, CNRS, UMR 5557, Ecologie Microbienne, Villeurbanne F-69622, France

In Arabidopsis, four homeotic gene classes, ABCE, are required for the patterning of floral organs. However, very little is known on how the activity of these master genes is translated into regulatory processes leading to specific growth patterns and the formation of organs with specific shapes and sizes. Previously we showed that the transcript variant *BPEp* encodes a basic helix-loop-helix (bHLH) transcription factor involved in limiting petal size by controlling post-mitotic cell expansion. *BPEp* is one of two transcripts originating from the *BIGPETAL* gene through an alternative splicing event which results into the fifth intron retention in *BPEp*. The second transcript (*BIGPETALub* or *BPEub*) is ubiquitously expressed. Furthermore, *BPEp* accumulation is regulated downstream of flower organ identity genes and this regulation is likely to be indirect requiring signaling intermediates and/or other co-factors.

We investigated on the one hand the molecular mechanisms implicated in the regulation of *BPEp* expression and on the other hand the interacting factors with *BPEp* during its function. Our data suggest that phytohormones are involved in the regulation of alternative splicing and intron retention in *BPEp*. Furthermore, during its function, *BPEp* interacts with the BiaF (*BPEp* interacting factor) transcription factor to limit cell expansion and thus petal growth at late development stages. Recent data on a novel pathway implicated in the regulation of petal growth will be discussed.

P133 TIME FOR COFFEE promotes the morning phase of the circadian clock by interacting with transcription factors

Jieun Shin, Alfredo Sanchez-Villarreal, Zhaojun Ding, Seth J Davis

Dept. of Plant Developmental Biology, Max-Planck-Institute for Plant Breeding Research, Koln, Germany

The light/dark cycle of one day provides an input signal to the circadian oscillator. *TIME FOR COFFEE (TIC*) is an important circadian-input factor inducing transcription of the morning core-oscillator gene, *LATE ELONGATED HYPOCOTYL (LHY)*. Furthermore, the tic mutant displays early periodicity in circadian rhythms. TIC protein has been shown to localize to the nucleus, however, its biochemical mechanism has not been identified. To investigate the underlying molecular mechanism of circadian-input signal involving TIC, we identified several TIC-binding partners through yeast-two-hybrid screening. Here we report that N-terminal fragment of TIC directly binds to two different transcription factors, TIC-BINDING PROTEIN1 (TBP1) and TIC-BINDING PROTEIN2 (TBP2). *TBP1* encodes a nuclear localizing MYB-domain protein, and *TBP2* encodes a bHLH motif protein. The transcript level of both genes is diurnally regulated with highest expression in the mid-day. We isolated mutations in *tbp1* and *tbp2* and generated mutants harboring a clock-promoter::luciferase reporter. In luciferase-imaging assay of these genotypes, *tbp1* and *tbp2* displayed a short periodicity. This phenotype is comparable to the tic mutant. In addition to promoter-reporter assay, we found that *tbp1* and *tbp2* mutants have phase-shifted *LHY* transcript accumulation, compared to the wild type. Therefore, we suggest that *TBP1* and *TBP2* can be intermediate signals between the dawn light inputs and the core oscillator.

P134 Transcription factor-hormone cross-talk in LEAFY COTYLEDON1 mediated embryogenesis

Astrid Junker, Twan Rutten, Gudrun Mönke, Helmut Bäumlein

Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany

The transcription factor (TF) LEAFY COTYLEDON1 (LEC1) acts as an essential regulator of Arabidopsis embryogenesis and seed development. It controls aspects of early embryogenesis like cotyledon identity and suspensor morphology, as well as seed maturation processes like storage compound accumulation and desiccation tolerance. Ectopic expression of LEC1 leads to the induction of somatic embryogenesis. Several upstream regulators of LEC1 have been identified, including chromatin remodelling factors like PKL, VAL/HIS and HDAC6/19, as well as other transcription factors like ASIL1 and MYB115/118.

To identify downstream components of the LEC1 regulon, we applied dexamethasone- and estradiol-regulated expression of LEC1 and describe the abscisic acid (ABA) dependent switch from vegetative growth to an embryonic developmental programme. Shoot apical meristem (SAM)-derived cotyledon-like leaves and embryo-like structures derived from the root apical meristem (RAM) are shown to store seed storage proteins, lipids and starch. LEC1-triggered embryonic differentiation seems to originate from stem cells in SAM and RAM leaving meristem identity unaffected. We describe a LEC1- and ABA-induced auxin maximum at the root-hypocotyl-junction which leads to callus formation and subsequent somatic embryogenesis.

Microarray and CHIP/chip analysis revealed the over-representation of hormone-related, lipid biosynthetic and transcription factor genes among putative LEC1 targets. The activation of auxin as well as brassinosteroid signalling by LEC1 supports its function during somatic embryogenesis and provides novel insights into TF-hormone cross-talk during early zygotic embryogenesis.

P135 A search for mutations suppressing the morphological phenotypes of *argonaute 1*

Verónica Aguilera, Patricia Quinto, Rosa Micol-Ponce, José Luis Micol, María Rosa Ponce

Division de Genetica and Instituto de Bioingenieria, Universidad Miguel Hernandez, Campus de Elche, 03202 Elche, Spain

ARGONAUTE (AGO) proteins are key components of the RNA-Induced Silencing Complex (RISC) that acts in the microRNA pathway. In *Arabidopsis thaliana*, the *ago1* loss-of-function mutant alleles of the *AGO1* gene disturb many developmental processes and often cause lethality or sterility.

With a view to identify novel genes involved in microRNA-guided gene silencing, we mutagenized seeds of the viable and fertile *ago1-52* line, already isolated in our laboratory. We have screened 36,810 M2 seeds, identifying 17 lines in which the morphological phenotype caused by *ago1-52* is partially or almost completely suppressed. We are positionally cloning these suppressor mutations.

P136 Regulation of *AHP6*, a main player in Arabidopsis root vascular development

Ana Campilho, Ykä Helariutta

University of Helsinki, Finland

In the Arabidopsis root, vascular cell identity is dependent on the localised inhibition of signalling of the phytohormone cytokinin by AHP6. Protoxylem cell fate is dependent on the localised inhibition of cytokinin signalling by AHP6, a pseudo-phosphotransfer protein that acts to inhibit the phosphorelay associated with cytokinin signalling. AHP6 is expressed specifically in both protoxylem cell files. The identity of either the negative regulatory or promotive factors which converge on *AHP6* is unknown. To identify and characterize upstream factors controlling *AHP6*, a forward genetic screen was performed to identify modified patterns of *AHP6::GFP* expression (*mae* mutants) within an EMS mutagenized line. Several mutants were identified and the phenotypical and molecular characterization of these loci will be presented. Further functional analysis of those loci can reveal basic genetic mechanisms underlying Arabidopsis vascular cell morphogenesis.

P137 Analysis of SCI1 - a novel Pc-G protein?

Mareike Hohenstatt, Daniel Schubert

Heinrich-Heine-University Duesseldorf, Germany

Polycomb-group (Pc-G) proteins are epigenetic regulators that play an important role in development and the control of cell fate of eukaryotic organisms. They confer mitotically heritable repression of target genes and are organized in high molecular weight complexes. Polycomb repressive complex 2 (PRC2) trimethylates lysine 27 of histone H3 (H3K27me3) which is bound and interpreted by PRC1. In plants, only PRC2 and H3K27me3 are conserved, thus it is unclear how H3K27me3 results in stable gene silencing.

We carried out a Y2H-screen with the Arabidopsis PRC2 member CLF (CURLY LEAF) to identify novel proteins involved in Pc-G function. One of the interactors, SCI1 (SWINGER/CURLY LEAF-INTERACTOR1), comprises a domain implicated in the binding of methylated lysines. SCI1 localizes to the nucleus and is expressed in various organs throughout the plant life cycle. Furthermore SCI1 is involved in the regulation of the floral repressor *FLC (FLOWERING LOCUS C),* one major Pc-G target. Due to a genetic and physical interaction with CLF and the regulation of *FLC* we assume that SCI1 could play a role in Pc-G mediated gene silencing.

P138 Divergent and redundant roles of the homologous BAH-PHD-containing proteins SHL and EBS in the regulation of developmental transitions in Arabidopsis

Leticia Lopez-Gonzalez, Laura Narro-Diego, Jose A Jarillo, Manuel Piñeiro

INIA-CBGP, Madrid, Spain

Chromatin remodeling factors can control the expression of a large number of genes and therefore, the duplication of genes encoding epigenetic regulators can contribute to the evolution of genetic networks required for proper control of plant developmental transitions. *EARLY BOLTING IN SHORT DAYS (EBS)* and *SHORT LIFE (SHL)* encode highly homologous nuclear proteins bearing two domains (BAH and PHD) frequently found in transcriptional regulators involved in chromatin remodelling. EBS is required to repress the floral integrator *FT* and is also involved in the regulation of other developmental processes such as seed dormancy in Arabidopsis. SHL is necessary for a proper development and fertility of Arabidopsis plants.

We are characterizing the role of this plant specific family of proteins in the regulation of developmental transitions. Expression analyses show that both *EBS* and *SHL* have similar patterns of expression, and loss-of-function alleles for *SHL* reveal a role for this locus in the repression of flowering. Genetic analyses indicate that the interaction of *SHL* with the Arabidopsis floral integrators *FT* and SOC1 is distinct from that of *EBS*. *SHL* appears to be required for *SOC1* but not *FT* repression, suggesting that both BAH-PHD proteins play independent roles in the control of flowering time. However, *ebs shl* double mutants display a strong induction of both floral integrators and extreme early flowering, suggesting that in addition to independent roles in the control of flowering, *EBS* and *SHL* can partially compensate each other´s function in the regulation of the floral integrators. Interestingly, *SHL* appears to be redundant with *EBS* in the repression of germination during seed dormancy, as shown by the phenotype of double mutants *ebs shl.*

Additionally, our results show that both proteins can bind histones; progress in understanding their histone binding properties will be also discussed.

P139 CLI1 - a novel component in cell fate determination

Nicole Schatlowski1, Justin Goodrich2, Daniel Schubert1

1Heinrich-Heine-University Duesseldorf, Germany, 2University of Edinburgh, UK

Plant cell fate is controlled by various epigenetic mechanisms with Polycomb-group (Pc-G) proteins as major regulators that maintain repressed transcriptional states of genes. Pc-G proteins regulate many aspects of plant development like seed development, flowering time and meristem identity. One Pc-G complex, Polycomb-repressive complex 2 (PRC2), has been shown to act as a histone methyltransferase (HMT) with activity towards lysine 9 and 27 of histone H3. These marks are correlated with stable, mitotically heritable repression of homeotic genes. Although PRC2 is conserved in plants, not much is known about proteins involved in Pc-G recruitment and stable repression of gene silencing.

In studies to identify novel factors involved in these processes the plant specific coiled-coiled protein CURLY LEAF INTERACTOR 1 (CLI1) was discovered. *cli1* mutants exhibit severe developmental defects including ectopic outgrowth on several tissues and reduced fertility. In addition, misexpression of Pc-G target genes suggests a role for CLI1 in Polycomb-mediated gene silencing. Interestingly, besides its occasional nuclear localisation CLI1 can also be found in different cellular compartments what might indicate multiple roles besides chromatin regulation.

P140 Identification of root vascular patterning mutants

Jan Dettmer, Robertas Ursache, Ana Camphilo, Ykä Helariutta

Institute of Biotechnology & Department of Biological and Environmental Sciences, University of Helsinki, Finland

The vasculature of the plant functions as a long distance transport system for water, nutrients, sugars and hormones. In the root the two conducting tissue types, phloem and xylem are arranged in a highly ordered pattern. To identify new genes involved in vascular tissue development, two different genetic screens based on ethyl methane sulfonate (EMS) mutagenesis have been performed. To identify genes involved in phloem development, mutants with alterd AtSUC2::GFP (a phloem marker) expression pattern were identified. The second screen was designed to isolate genes acting up- or downstream of AHP6, an inhibitory pseudophosphotransfer protein that counteracts cytokinin signaling thereby allowing protoxylem formation. Several mutants with AHP6::GFP misexpression were isolated.

Here we describe the isolation and characterization of three mutants with similar phenotypes. The mutants have a short root with a disorganized stele pattern and an increased number of phloem cells. The similar stele phenotypes strongly suggest that the three genes acting on the same patterning process within the stele.

P141 The role of APL as a transcriptional regulator in specifying vascular tissue identity

Raffael Lichtenberger, Satu Lehesranta, Ove Lindgren, Sari Tähtiharju, Ykä Helariutta

Institute of Biotechnology/Department of Biological and Environmental Sciences, University of Helsinki, Finland

The vascular system of higher plants confers efficient conduction and provides mechanical support. It consists of two kinds of conducting tissues, xylem and phloem. Phloem transports the products of photosynthesis and provides paths for translocation of proteins and mRNAs involved in plant growth and development. Although there are some reports of gene expression characteristic to phloem, the molecular basis of phloem development is still largely unknown. The APL transcription factor (Altered Phloem Development) was identified as the first gene specifying vascular tissue identity. Based on cell sorting coupled with genome-wide microarray analysis, we have been able to uncover phloem abundant regulatory genes dependent on APL. The results indicate that APL is a key node for transcriptional activation of gene expression characteristic to phloem development and for transcriptional repression of gene expression characteristic to xylem development. We are currently studying the possible functions of the identified genes in phloem development.

P142 SKP2A, an F-box protein that regulates cell division

Silvia Jurado, Zamira Abraham, Carlos del Pozo

Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Madrid, Spain

Coordination between cell division and cell differentiation is crucial for growth and development of eukaryotic organisms. Progression through the different phases of cell division requires the specific degradation of proteins through the ubiquitin-proteasome 26S pathway (Ub/26S). In plants, this pathway plays a key role in controlling several developmental processes and responses, including cell proliferation. Ubiquitin is attached to target proteins in sequential biochemical cascade that involves the E1, E2 and E3 enzymes. There are different types of E3. One of these types is the SCF complex, which is composed of 4 protein subunits, CUL1, RBX, ASK1 and an F-box. SKP2A is an F-box protein forms an SCF complex *in vivo* that has E3 ubiquitin ligase activity. SKP2A regulates the stability of the cell division E2FC-DPB repressor transcription factor, and subsequently positively regulates cell division. Plants that over-express SKP2A increase the number of cells in G2/M, reduce the level of ploidy and develop higher number of lateral root primordia. Interestingly, SKP2A is degraded through the Ub/26S pathway and auxin regulates such degradation. We have found that auxin is capable of regulating SKP2A stability in a cell free system, suggesting that the regulation of auxin is direct. Taken together, our results indicate that SKP2A is a positive regulator of cell division and its stability is controlled by auxin-dependent degradation.

P143 Identification of novel factors involved in vascular development

Katrin Ollram, Thomas Greb

Gregor Mendel Institute, Vienna, Austria

A continuous network of vascular bundles, consisting of xylem and phloem, pervades every organ of the plant. The xylem is responsible for the transport of water and nutrients, while the phloem transports sugars, proteins, RNA and other signalling molecules. The establishment of these specialised and interconnected cell types requires highly organised cell differentiation and tissue patterning. However, despite its fundamental role in many aspects of plant growth and physiology, the molecular regulation of this complex process is still elusive.

The MYB transcription factor *ALTERED PHLOEM DEVELOPMENT (APL)*, is the only known cell identity gene required for the differentiation and maintenance of a vascular tissue, in this case the phloem.[1] In order to identify novel factors required for the differentiation and specification of vascular tissues in general, and phloem in particular, a mutagenesis screen was designed based on transgenic Arabidopsis lines carrying a *pAPL:LUCIFERASE (LUC)* reporter gene. Mutations in positive and negative regulators of the *APL* gene are expected to reduce or enhance the LUC-signal, respectively. This approach is complemented by analysing lines carrying different *APL* promoter-deletion constructs with regard to the strength and pattern of mediated gene expression.

Our approaches allowed us to identify mutants representing putative (novel) regulators involved in cell specification processes during vascular development. One of them, the reduced in *apl:luc signal1 (ria1)* mutant displays a stunted growth habit and seedling lethality suggesting that an essential factor is affected. The characterisation of this and other identified factors and promoter elements will contribute to our understanding of molecular mechanisms important for vascular development in plants.

Support: Austrian Academy of Sciences, Austrian Science Fund (FWF)

Bonke M, Thitamadee S, Mähönen AP, Hauser M-T and Helariutta Y (2003) APL regulates vascular tissue identity in Arabidopsis. Nature 426:181-186.

P144 Over expression of a plant homolog of the human tumor suppressor PTEN leads to flower sterility

Rodnay Sormani1,2, Anne Pribat1, Mathieu Rousseau3, Ludivine Taconnat4, Jean Pierre Renou4, Christian Meyer2, Christophe Rothan1, Véronique Germain1

1UMR 619 Biologie du Fruit, INRA Bordeaux, France, 2Laboratoire de Nutrition Azotee des Plantes, IJPB, INRA Versailles, France, 3Unite de Recherche sur les Especes Fruitieres, INRA Bordeaux, France, 4Unite de Recherche en Genomique Vegetale, INRA Evry, France

In animals, PTEN (Phosphatase and TENsin homolog) acts as a negative regulator of the PI3K pathway converting the second messenger phosphatidylinositol PI(3,4,5)P3 to PI(4,5)P2. In plant, phylogenetic analyses have revealed that *PTEN* genes have evolved into two separate groups distinct from animal PTEN: the *PTEN1* group, and the *PTEN2* group found in all plant taxa analysed to date, from green algae to eudicots. In Arabidopsis, 3 genes coding for *PTEN* homologs do exist. *PTEN1* clade is represented by only one gene already characterised in Arabidopsis. The *PTEN2* clade includes two genes named *PTEN2A* and *PTEN2B*. Overexpression of *PTEN2A* had no effect during vegetative growth under normal conditions but led to a severe default during reproductive development. Sterile flowers of normal size and shape were produced and, as a consequence, small parthenocarpic siliques were observed on the plant. Pollination with WT pollen led to normal silique development. The flower sterility previously described for the *PTEN1* RNAi lines was due to a default in pollen development (Gupta *et al* Plant Cell 2002). Here, the observed default seems to be male sterility too but, in this case, it is correlated with *PTEN2A* over accumulation. The PTEN2A-induced male sterility is associated with the lack of endotheticum break during stamen dehiscence and with reduced callose deposition in the stamen of sterile flowers. DNA chip transcriptome comparison of sterile and fertile flowers showed the down-regulation in sterile flowers of genes implicated in stamen development. It further highlighted the possible role of hormones signalling in this process. All together, these results suggest a role for *PTEN2A* in plant development in interaction with hormone signalling.

P145 Functional characterization of Metacaspase 9 in *Arabidopsis thaliana*

Sunil Kumar Singh, Benjamin Bollhöner, Luis Muñiz, Hannele Tuominen

Umea Plant Science Centre, Department of Plant Physiology, Umea University, Umea, Sweden

Xylem cell death is a crucial developmental process during xylem tissue differentiation in plants. Key regulators of programmed cell death (PCD) in animals are a family of proteases, called caspases. Caspase homologues could not be found in plants, but a family of distant structural caspase relatives was discovered, the metacaspases. Metacaspases are thought to be involved in plant PCD and recent studies indicate a function of plant metacaspases in developmental and UVC-mediated PCD. Based on gene expression analyses, we have shown that two poplar metacaspases are specifically upregulated during the xylem cell death phase of wood formation, indicating a function in xylem PCD.

Arabidopsis has nine metacaspase genes, the closest homologue to the two xylem-specific poplar genes is metacaspase 9 (*AtMC9*; At5g04200). Reporter gene analysis showed that *AtMC9* is specifically expressed in xylem vessels of root, stem, hypocotyl, cotyledons, sepals and petals, root cap cells and the connective tissue between anther and filaments. To characterize the role of AtMC9 during plant development, several T-DNA insertion lines have been identified. These lines did not show a strong visible phenotype, which might be due to functional redundancy between different members of the

metacaspase family. Therefore, we have generated several RNA interference (RNAi) and double knock-out mutant lines. RNAi lines showed significant suppression of *AtMC9* expression as well as suppression of several other metacaspases, resulting in a range of developmental phenotypes including larger stem and rosette leaves, increased size of vascular bundles, longer roots and various flower abnormalities, which all indicating an increase in meristem size in these RNAi lines. Hence, we suggest that *AtMC9* together with one or several other metacaspases function in determining the sizes of the meristems. To further characterize these phenotypes, we have crossed the RNAi and knock-out mutant lines with various markers that are expected to reveal the developmental processes affected in these lines.

P146 Investigation of the physiological function of sink-related sucrose transporters in *Arabidopsis thaliana*

Bianca Schmitt, Inga Barth, Stefan Meyer, Norbert Sauer

Molecular Plant Physiology, University of Erlangen-Nuremberg, Germany

In *Arabidopsis thaliana* the sucrose transporter gene family consists of nine members, *AtSUC1-AtSUC9*, with high sequence homologies up to 96% identity within the family.

AtSUC2 could be characterized as the principal phloem loading transporter on the basis of localization studies and uptake analyses. Genetic evidence for its crucial function was given by the analysis of T-DNA insertion lines displaying retarded development and sterility due to the impaired photoassimilate partitioning.

In contrast to the phloem loader *SUC2* the other sucrose transporter genes are mainly expressed in sink tissues such as flowers and roots or were identified as pseudogenes *(SUC6, SUC7).*

Until now mutant analyses of sink localized sucrose transporters did not show any or only weak phenotypes, but revealed first genetic insights into the physiological roles in the respective tissues. *AtSUC5* T-DNA insertion lines, for example, showed alterations in the fatty acid composition of the endosperm and in regard to the endosperm specific expression of *AtSUC5* a role in seed development was proposed. In addition, *AtSUC9* mutant analyses showed a slight variance in flowering time. For *AtSUC1* a function in anther dehiscence and sucrose signaling has been discussed, depending on the expression and localization data and the analysis of T-DNA insertion lines. So far, for *AtSUC3* and *AtSUC8* no genetic evidence for their physiological roles could be investigated to date.

For *SUC1, SUC3, SUC5, SUC8* and *SUC9* expression and localization in floral tissues has been shown. Considering these data and the high sequence homologies within the Arabidopsis sucrose transporter gene family redundant functions of these proteins might be possible. To address the genetic evidence for the physiological roles of the sink related Arabidopsis sucrose transporters we successfully created quadruple and quintuple insertion lines affected in all five genes. Detailed analyses of the floral, embryo and seed development will be performed, anticipating more severe phenotypes especially in the reproductive tissue. Here we present a first look at the phenotypes of these plants.

P147 Novel role for a pair of NAC-like genes in regulating shoot architecture in Arabidopsis

Ching-Fang Shih, Jian-Shin Lin, Chang-Hsien Yang

Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan

NAC-like genes that contained a conserved 150 amino acid *NAC* domain at their N-terminus of proteins have been thought to be involved in regulation of many processes of plant development. *AtNACL8* and *AtNACL10*, two consecutive NAC-like genes on chromosome 3 with 84% protein sequence identity, were identified in Arabidopsis. Both *AtNACL8* and *AtNACL10* mRNA were detected in the 7-, 14, and 21-day-old plants and in cauline leaves, inflorescence and flowers of a mature plant. Similar pattern of GUS activity was detected in the cotyledons, roots and young leaves of AtNACL8::GUS and AtNACL10::GUS transgenic Arabidopsis'plants. GUS activity was also specifically detected in shoot apical meristem (SAM) and axillary meristem regions of the inflorescence. Transgenic Arabidopsis plants ectopically expressed *AtNACL8* or *AtNACL10* were phenotypically indistinguishable from wild-type plants. By contrast, shoot formation was severely altered in antisense mutants of *AtNACL8* and *AtNACL10* by producing multiple branches with secondary inflorescence or flowers in the same position of an internode. Interestingly, a much severe alteration of the shoot formation by producing up to six branches was observed in *AtNACL8/AtNACL10* double mutants generated by RNAi strategy. Further analysis indicated that the mutant phenotype was correlated with the down-regulation of *FASCIATA1 (FAS1), FAS2* and up-regulation of *WUSCHEL (WUS)* in mutants of *AtNACL8* and *AtNACL10*. These results indicated that *AtNACL8* and *AtNACL10* are likely the homologues with redundant function in controlling meristematic activity and shoot formation by regulating the expression of *FAS* and *WUS*.

P148 GIGANTEA mediates senescence and oxidative response in plants

Yu-Yun Chang, Ya-Chun Li, Ching-Fang Shih, Emily Chin-Fun Chen, Muthu Thiruvengadam, Chang-Hsien Yang

Graduate Institute of Biotechnology, National Chung Hsing University, Taichung, Taiwan 40227 ROC

The senescence of detached leaves was significantly delayed in *gigantea (gi)* mutants of Arabidopsis and Brassica. The result also showed that the strength of delay of leaf senescence was correlated to the strength of delay of flowering in gi mutants of Arabidopsis. Ectopic expression of Arabidoipsis *GI* in *gi-1* mutant caused leaf senescence even faster than wild-type leaves. This result indicated that increasing of *GI* expression could promote not only

flowering time but also leaf senescence. Further analysis indicated that the delay of leaf senescence was associated with the up-regulation of *CATALASE3 (CAT3).* The induction of this gene in *gi* mutants also greatly enhanced the resistance to the infection by *Ralstonia solanacearum* and the tolerance to chilling stress. Furthermore, genes involved in stress response such as *low temperature- induced protein 78 (LTI/COR78) (At5g52310), dehydrin (ERD10) (At1g20450)*, and *membrane channel protein (At2g28900)* were up-regulated in *gi-1* mutants by microarray analysis. Our results revealed that GI protein is functionally important in flowering as well as in response to photoperiodic and oxidative stimulations in plants.

P149 The flower development size-clock

Susana Sauret-Gueto1, Jerome Avondo2, Andrew Bangham2, Enrico Coen1

1Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH UK, 2School of Computing Sciences, University of East Anglia, Norwich, NR4 7TJ UK

Floral organs arise as undifferentiated primordia that grow and differentiate into one of the four types of mature organs: sepals, petals, stamens and carpels. In the last two decades we have begun to understand how genes products control the mechanisms of flower development, from the identity, number and positioning of floral organs in each whorl to the termination of the floral meristem. If we want to understand how gene activity is translated into a morphological form we need to address growth as a dynamic process and introduce the time variability. Thus, we have extended Smyth *et al* 1990 description of landmarks of Arabidopsis flower development into a quantitative staging system. This would allow the Arabidopsis community to have a quantitative framework of flower development in order to analyse the organogenesis of floral organs, to relate gene products activities to the control of growth and to construct mechanistic models. To gather the data we have used Optical Projection Tomography (OPT) and the Volviewer software package for capturing and measuring 3D data from plant inflorescences (Lee *et al*, 2006).

In addition, as petals are increasingly being used as a model for plant organogenesis given their relative simplicity, we have also generated a growth curve for the petal that will be used to generate a petal model and test different genes control of petal growth.

P150 Functional analysis of a Trithorax group (TrxG) gene *ASHH1* in *Arabidopsis thaliana*

Ya-Chun Li, Chun-Yuan Lin, Chang-Hsien Yang

Graduate Institute of Biotechnology, National Chung Hsing University, Taiwan

During development and cell differentiation, chromatin remodeling is essential for the reprogramming of the transcriptional control of gene expression. In Drosophila, this process was regulated by the antagonistic interaction between two groups of chromatin factors, the Polycomb group (PcG) and the Trithorax Group (TrxG). In general, PcG proteins repress gene expression whereas trxG proteins counteract the effect of the PcG proteins. We study one Arabidopsis trxG gene *ASHH1* which contains the SET domain and is a protein lysine methyltransferase enzyme. The mRNA expression of *ASHH1* was detected in plants at different developmental stages and various organs. Its expression was increased through the development. The flowering time was delayed and the leaf development was affected in ashh1 mutants. Further analysis indicated the expression of *FT* was down-regulated whereas *FLC* was up-regulated in ashh1 mutants. This result indicated that *ASHH1* is required for the flower transition in both photoperiod flowering pathway and vernalization pathway. In addition, leaf senescence was significantly delayed in *ashh1* mutants and was correlated with the down-regulation of senescence-associated genes, *SAG12, SEN1, WRKY6*, and *WRKY53*. Our data reveals that ASHH1 regulates the flowering time, leaf formation and senescence of Arabidopsis by modulating histone methylation and gene activity.

P151 Phosphoinositides system in Arabidopsis flower development

Kazue Kanehara1, Yuki Nakamura1, Zhi Wei Teo1, Wei Fun Cheong2, Guanghou Shui2, Siou Ting Gan1, Markus R Wenk 2,3, Toshiro Ito1

1Temasek Life Sciences Laboratory, Singapore, 2Department of Biochemistry, National University of Singapore, Singapore, 3Department of Biological Sciences, National University of Singapore, Singapore

Phosphoinositides (PIPn) are well known as lipid signaling molecules in numerous kinds of organisms. In Arabidopsis, PIPn signaling pathways are stimulated upon various environmental cues such as biotic stresses, elicitor responses, and different developmental aspects including root tip growth and reproductive process. Little is known about an involvement of PIPn system in reproductive process because of its complexity. Nevertheless, some of biochemical studies proved unusually high level of PI and active turnovers of PIPn metabolism in plant reproductive organs. Furthermore, recent gene knock out studies suggest the involvement of PIPn system in reproductive process. To explore PIPn system in flower development, we performed global profiling of PIPn species as well as gene expression possibly involved in PIPn metabolism by means of developmentally synchronized Arabidopsis flowers. Our results show dramatic changes in PIPn level and relevant gene expression during the course of flower development. This suggests that PIPn system plays an important role during flower development.

P152 A putative CHD3 chromatin remodeling gene implicated in seed maturation and germination

Nirmala Sharma, Yarnel Bender, Maureen Anderson, Kerry Sproule, Kasi Williams, Pierre Fobert

Plant Biotechnology Institute, NRC, Saskatoon, SK, Canada

CHD3 is a subfamily of the SWI/SNF DNA-dependent ATPases that use energy from ATP to remodel chromatin without covalent modifications. The family is highly

conserved throughout eukaryotes and has been studied intensely in yeast and animals where CHD3 proteins are found in complexes with histone deacetylase activity that repress transcription and embryonic programs during developmental transitions. We have characterized an Arabidopsis gene encoding a putative CHD3 chromatin remodeling protein using forward and reverse genetics approaches. We show that this gene is involved in regulating oil disposition in seeds and in mediating ABA responses in germinating seeds and drought responses in juvenile plants. Seed oil content in several independent transgenic lines was elevated compared to the wild type, whereas opposite phenotype was observed for a T-DNA insertion mutant in this gene. In addition to producing more seed oil, transgenic lines also germinated faster and better in presence of ABA than the wild type, whereas the mutant line was hypersensitive to ABA and hence displayed delayed or poor germination compared to its wild type. The mechanisms by which this gene regulates seed maturation and abiotic stress responses are being investigated.

P153 Insights into the *Arabidopsis thaliana* trehalose-6-phosphate phosphatase family

L López1, L Vandesteene1, T Beeckman2, P Van Dijck1

1Department of Molecular Microbiology, VIB-K.U.Leuven, Belgium, 2Department of Plant System Biology, VIB-U.Ghent, Belgium

Trehalose is a disaccharide widely distributed in nature playing an important role in carbohydrate storage and stress protection. The major pathway in plants consists of two enzymatic reactions, the first reaction involves trehalose phosphate synthases (TPS) which convert UDP-glucose and Glucose-6-phosphate to uridine diphosphate (UDP) and α,α–trehalose-6-phosphate (T-6-P), and in the second step T-6-P is de-phosphorylated by trehalose phosphate phosphatases (TPP) to produce trehalose and inorganic phosphate. Trehalose was thought to be absent in plants, but genome sequencing and mutant analysis using ectopic expression of microbial trehalose genes showed that trehalose metabolism is essential for normal growth and development as impressive phenotypes related to sugar partitioning, carbon allocation and stress resistance were found. These results are more associated to the change in the level of the intermediate T-6-P, which is strongly related to the sugar status of the plant, than to the trehalose itself, showing the importance of the T-6-P as a novel coordinator of sugars in plants. Our work is being focused in the AtTPP multigene family (*TPPA-J*) which conserves the phosphate boxes of the yeast TPP (Tps2) enzyme. These genes are active phosphatases able to complement the growth phenotype of the yeast tps2 mutant and they showed *in vivo* TPP activity. Marker lines have been developed to identify cell-type of the promoter activity and revealed specific expression patterns of these genes in cotyledons, leaves, roots and flowers. Our research is mainly focused in those TPPs present in the root, mutant analysis has revealed arrested root growth and decreased number of lateral roots with a shorter root meristem. Preliminary results show that auxin can complement these phenotype by increasing the number of lateral root primordia in the mutant however there is a delay in the lateral root outgrowth, event that is still under investigation. More experiments as measurement of sugars, trehalose and the intermediate T-6-P levels are needed to better understand the role of these genes in sugar signaling/metabolism and plant growth.

P154 MicroRNA profiling of *Arabidopsis thaliana* mature pollen

Gael Le Trionnaire1, Robert Grant-Downton2, Said Hafidh1, Ralf Schmid3, Hugh Dickinson2, David Twell1

1Dept of Biology, University of Leicester, 2Dept of Plant Science, University of Oxford, 3Dept of Biochemistry, University of Leicester

Angiosperm gametophytes are haploid structures composed of a few cells embedded in sporophytic tissues. The male gametophyte (or pollen grain) is composed of two sperm cells and a single vegetative cell that will deliver the gametes via the pollen tube to the embryo sac for double fertilisation. The pathway leading to their formation starts from a uninucleate microspore that asymmetrically divides into a bicellular pollen grain composed of a large vegetative cell and a small germ cell, which then divides to form two sperm cells present in the mature tricellular pollen grain. The transcriptomic profile of these developmental stages has already been extensively characterised.[1]

Small RNAs are also essential components of the transcriptome, both to protect the genome against transposons or aberrant nucleic acids (siRNAs) and regulate gene expression (miRNAs). Recent studies carried out at mature pollen stage confirmed that small RNA pathways were functional[2] and exhaustive identification of small RNAs suggested that an epigenetic reprogramming occurred through silencing of transposons by siRNAs.[3] Nevertheless, little is known about the involvement of miRNAs during pollen development. We thus used high-throughput sequencing approaches to detect these small RNAs in mature *Arabidopsis thaliana* male gametophyte.

We sequenced more than 50,000 small RNAs in Arabidopsis mature pollen using 454 technology. This analysis ensured the identification of nearly all miRNAs present at this developmental stage. We also discovered novel putative miRNAs using bioinformatic prediction tools and managed to validate these functionally by detecting the expected cleavage products using 5'-RACE-PCR. We also detected some transcripts showing trans-acting siRNA features. This study constitutes the first survey of miRNAs and putative ta-siRNAs in mature pollen and thus provides new insight into the molecular mechanisms regulating male gamete formation in flowering plants.

1 Honys and Twell. 2004 Genome Biol, 5:R85.
2 Grant-Downton *et al* 2009 Mol Plant, 1:1-13.
3 Slotkin *et al* 2009 Cell, 136:461-472.

P155 *duo pollen 4* – a novel gametophytic mutant blocking male germ cell division in Arabidopsis

Hoda Khatab, Tony Wardle, David Twell
Dept of Biology, University of Leicester, UK

In flowering plants, the formation of two sperm cells plays a critical role in double fertilisation and plant fertility. Despite the importance of sperm cells in plant reproduction, relatively little is known about the molecular mechanisms that govern sperm cell formation. To uncover these mechanisms, six independent gametophytic division mutants were isolated in a pollen morphological screen of an EMS-mutagenised Arabidopsis population. The mutants, termed *duo pollen (duo),* specifically affect germ cell division resulting in pollen containing a single germ cell at maturity.[1] The *DUO1* gene encodes a novel R2R3-type MYB transcription factor that is specifically expressed in the male germline[2] and provides a regulatory link between cell cycle progression and germ cell specification.[3]

Here we describe the *duo4* mutant, which like other *duo* mutants, is essential for sperm cell formation but has a distinct nuclear morphology. The single germ cell in *duo4* appears frequently elongated with non-uniform chromatin condensation. Genetic analyses revealed that *duo4* acts gametophytically and reciprocal crosses show that the *duo4* allele is transmitted normally through the female but there is no male transmission. Developmental analysis and nuclear DNA content measurements show that the *duo4* germ cell undergoes complete DNA replication but fails to enter mitosis. However, a small proportion of *duo4* germ cells are delayed in cell cycle progression and divide in the latter stages of pollen development, showing segregation defects. *duo4* pollen grains correctly express vegetative and germ cell fate markers, indicating that specification is maintained in the absence of germ cell division.

The *duo4* mutation has been localised on chromosome IV to a genetic interval of ~25 kb. Further studies on this mutant and isolation of the *DUO4* gene will provide valuable insight into the mechanisms controlling germ cell proliferation and specification.

1 Durbarry *et al* (2005) Plant Physol. 137:297-307.
2 Rotman *et al* (2005) Curr Biol. 15(3):244-248.
3 Brownfield *et al* (2009) PLoS Genet. 5(3):e1000430.

P156 Tissue layer-specific rescue of organ form in Arabidopsis revealed by the cell autonomous action of ANGUSTIFOLIA

Yang Bai1, Stefanie Falk1, Arp Schnittger2, Marc Jakoby1, Elena Galiana Jaime1, Martin Hulskamp1
1University of Koln, Botanical Institute III, Gyrhofstr. 15, 50931 Koln, Germany, 2Institut de Biologie Moleculaire des Plantes du CNRS, IBMP-CNRS - UPR2357 12, rue du General Zimmer, 67084 STRASBOURG, France

The shape of an organism is genetically determined, but how plants or animals reach their final shape is largely unknown. In higher plants, proper organ development is controlled by different tissue layers, epidermis (L1), subepidermis (L2) and vascular system (L3). For many years researchers have discussed and analysed the question which tissue layer determines organ shape. To address this question, we introduce *ANGUSTIFOLIA (AN)* to the *angustifolia* mutant *(an)* with tissue specific promoters. The narrow leaf phenotype is rescued by subepidermal expression of *AN*, and the epidermis expression of *AN* can rescue the petal and silique phenotype. We conclude that the shape of organs is not always controlled by one tissue layer but controlled by different tissue layers in different organs.

P157 *PIGGYBACK6* is required for apical embryo patterning in Arabidopsis

Dora Szakonyi, Mary Byrne
John Innes Centre, Norwich, UK

In the shoot, lateral determinate organs derive from the pluripotent proliferating cells of the apical meristem. During leaf development emergence of a new primordium depends on following a strict genetic program. Building up dorsal (adaxial) and ventral (abaxial) cell layers is one of the key events in this process and essential for forming a flat lamina. Recently *piggyback (pgy)* mutants were isolated as factors involved in dorsoventral patterning (Pinon *et al*, 2008). These recessive mutants show mild leaf phenotype including pointed leaf shape with enhanced serration and in combination with *asymmetric leaves1 (as1)*, develop adaxial ectopic leaf lamina outgrowths. Based on genetic interaction *PGYs* are modifiers of the HD-ZIPIII-KANADI pathway. *PGY* genes were shown to encode cytoplasmic large subunit ribosomal proteins. Here we report isolation of a semidominant *pgy* allele. Heterozygous *pgy6-1d* plants show the characteristic *pgy* leaf shape while homozygous plants have a more severe phenotype. This work is focusing on characterizing the observed mutant phenotypes with special attention to embryo development. Position of the point mutation in *pgy6-1d* was identified in another component of the large ribosome subunit.

Pinon V, Etchells JP, Rossignol P, Collier SA, Arroyo JM, Martienssen RA, Byrne ME. Three *PIGGYBACK* genes that specifically influence leaf patterning encode ribosomal proteins. Development. 2008 Apr;135(7):1315-24

P158 In search of interaction partners for 14-3-3 protein epsilon

Christian Throm
ZMBP, University of Tübingen, Germany

14-3-3 proteins constitute a family of well conserved proteins interacting with a large number of phosphorylated binding partners in eukaryotes. The major native forms of 14-3-3s are homo- and hetero-dimers, the biological functions of which are to interact physically with specific client proteins and thereby induce a change in the activity state of the client. As a result, 14-3-3s are involved in a vast array of processes such as primary metabolism, signal transduction and gene expression, serving as adapters, activators, and repressors. Plant development and seed germination appear also to be under control of factors whose interaction with 14-3-3 molecules is crucial for their activation.

The aim of this project is to identify proteins, which interact with the 14-3-3 isoform epsilon, which seems to be the ancient ancestral isoform. Therefore we performed a yeast-two-hybrid-screen using a cDNA library obtained from etiolated seedlings. We identified 378 proteins as putative interaction partners of the isoform epsilon. 16% of these proteins are involved in signal transduction, 55% function in metabolism and 11% play a role in basal processes of transcription and translation.

Because of the vast number of putative interaction partners, we focused on specific proteins. One putative interaction partner is BEE3, a basic helix-loop-helix (bHLH) transcription factor, which is an early response gene required for full BR response1.

Another one is FBR12, the translation initiation factor 5A-2 which plays a crucial role in plant growth and development.

The interaction studies will be verified via bimolecular fluorescence complementation (BiFC). Beyond, the focus lies on the identification of a 14-3-3 binding motif and the analysis of the biological relevance of significant interactions.

1 Friedrichsen D. M. *et al* Three Redundant Brassinosteroid Early Response Genes Encode Putative bHLH Transcription Factors Required for Normal Growth Genetics, Nov 2002; 162: 1445

2 Feng H. *et al* Functional Characterization of the Arabidopsis Eukaryotic Translation Initiation Factor 5A-2 That Plays a Crucial Role in Plant Growth and Development by Regulating Cell Division, Cell Growth, and Cell DeathPlant Physiology, Jul 2007; 144: 1531 – 1545

P159 TEM analysis reveals plasmodesmata structure is important for function

Tessa Burch-Smith, Patricia Zambryski

Department of Plant & Microbial Biology, University of California, Berkeley, CA, USA

In plants cell-to-cell communication is accomplished via cytoplasmic channels called plasmodesmata (PD). In addition to small molecules and nucleic acids, developmentally important molecules including transcription factors also move through PD. The upper limit of the size of molecules PD traffic (size exclusion limit, SEL) is developmentally regulated. Torpedo stage-Arabidopsis embryos undergo a transition that results in a reduced size exclusion limit; the ability to transport 10-kDa but not 0.5-kDa fluorescent tracers is lost. Further, the structure of PD is also developmentally regulated. In young leaves, PD are almost exclusively simple, straight channels but older leaves contain highly branched, elaborate structures. A screen in our lab identified several mutants that could transport 10-kDa tracers post the mid torpedo stage. Two of these mutants, *increased size exclusion limit (ise)1 and2,* encode essential RNA helicases. Interestingly, both *ise1* and *ise2* mutants display PD that are modified forms of the simple PD typically found in wild type embryos. The nature of relationship between the structure of PD and their SEL is not clear and it is intriguing to speculate that modification of structure would be one mechanism that could be used to regulate PD transport. Here we report the results of extensive transmission electron microscopic studies on different stages of wild type, *ise1* and *ise2* embryos in an attempt to examine the relationship between PD form and function. In contrast to what has been previously reported, our TEM analyses reveal that embryos contain not only simple PD but also twinned and branched forms. However, the modification remains under developmental control. Also surprising is the difference in modification observed in *ise1* and *ise2* embryos. Our data also reveal insights into the mid-torpedo down regulation of PD transport observed in wild type embryos.

P160 Functional redundancy and spatial expression of the *FLOWERING LOCUS T (FT)/TERMINAL FLOWER 1 (TFL1)* gene family in *Arabidopsis thaliana*

Seong Jeon Yoo, Kyung Sook Chung, Hanna Lee, Ji Hoon Ahn

Korea University, Seoul, South Korea

In Arabidopsis, *FLOWERING LOCUS T (FT)/TERMINAL FLOWER 1 (TFL1)* family is a small gene family that encodes phosphatidylethanolamine binding protein (PEBP). They have highly conserved sequence and are divided into two groups, each group have antagonistic function in flowering time and meristem identity, as *FT*-like genes and *TFL1*-like genes. We investigated the redundant effect on each two groups by genetic analysis. *ft-10* show late flowering time and late determination, *tfl1* mutants show early termination and early flowering time. However, other mutants did not have any flowering time and meristem determination phenotype. Interestingly, *tfl1 bft-2* atc-2 shows more strong terminal flower phenotype, they have short primary stem length and fewer solitary flowers and accelerate flowering time than *tfl1-20* single mutants. Also *ft-10 tsf-1* double mutant and *ft-10 mft-2* double mutant show more late flowering than *ft-10* single mutants and late determinate of apical meristem. We think that *FT/TFL1*-like genes have redundant function of *FT* or *TFL1* in flowering time and meristem development.

P161 Plant specific signalling outputs of the TOR kinase growth regulatory pathway

Natasha Forester, Karen Wilson, Bruce Veit

AgResearch, Palmerston North, New Zealand

To gain insight into basic aspects of growth regulation in plants, we have begun an analysis of the TOR pathway, an ancient eukaryotic regulatory pathway. Named for a key growth enabling kinase, TOR (Target of Rapamycin), the pathway has been analysed extensively in fungi, where its growth promoting activity was first genetically characterised, and in metazoans, where its regulation features in both normal and malignant forms of growth.[1] More recent studies suggest certain aspects of TOR regulation are shared by plants, including a close coupling between TOR activity and growth,[2,3] and the up-regulation of translational capacity through TOR activation of S6K (S6 Kinase).[4] Activation of S6K by TOR appears to depend on a scaffold-like interaction with RAPTOR, a conserved WD-40 repeat protein. While RAPTOR targeted

phosphorylation of S6K appears common to all eukaryotic groups, other RAPTOR targeted substrates may be less well conserved. We discuss evidence that suggests that certain aspects of TOR signalling may be specific to plants, including mechanisms that regulate the activity of RAPTOR,[5], as well as its interaction with Mei2-like RNA proteins,[6,7] which are involved in growth regulation.

1 Cell, 2006. 124: p. 471-484.
2 EMBO Rep, 2007. 8(9): p. 864-70.
3 Proc Natl Acad Sci U S A, 2002. 99(9): p. 6422-7.
4 Plant Cell, 2006. 18(2): p. 477-490.
5 Mol Cell, 2008. 30(2): p. 214-26.
6 Plant Mol Biol, 2004. 54(5): p. 653-70.
7 BMC Plant Biol, 2005. 5(1): p. 2.

P162 Live-organ-imaging of auxin responses with the luciferase reporter gene

Masaaki K Watahiki, Kotaro T Yamamoto

Hokkaido University, Faculty of Science, Sapporo, Japan

Auxin plays intriguing roles for plant organogenesis. The auxin-insensitive mutants, *nph4/massugu1/arf7* and *massugu2/iaa19* are isolated by hypocotyl curvature test (Tatematsu *et al*, 2004, Watahiki and Yamamoto., 1996). Like as the other auxin related mutants, *massugu2* or *arf7/arf19* double mutant (Okushima *et al*, 2007) show organ specific defects, i.e. less lateral roots or aberrant leaf shape. To investigate the initiation and development of lateral organ, the live imaging technique is applied for monitoring IAA19 expression.

IAA19 promoter activity was observed by three reporter genes, green fluorescent protein (GFP), beta-glucronidase (GUS) and emerald luciferase (ELuc). Although all reporters showed similar spatial expression pattern, pIAA19::GFP made the best spatial resolution in the root with confocal microscope, pIAA19::GUS enable the visualization of thick part of the tissue by fixation and vitrification and pIAA19::ELuc made the least damage for live tissue. Application of exogenous auxin to root increased the signal of three reporters, pIAA19::ELuc respond less than 30 min which was the fastest among them. This result suggests that the velocity of ELuc protein maturation is the fastest. pIAA19::ELuc plant shows spot like signals along with the primary root. Some of the signals were weaken during the growth of the root but the other stayed the level of the signal and emerged the lateral root. These spot like signals on the primary root suggests the candidate loci for lateral root formation and with considering less lateral root phenotype in *massugu2*, IAA19 function implies the role for a determination of lateral root among the candidate loci. We report that a spatial and temporal action of auxin during lateral organogenesis.

P163 The *MAB/ENP* family genes involved in auxin-regulated morphogenesis

Masahiko Furutani, Shuhei Yoshida, Masao Tasaka

Nara Institute of Science and Technology (NAIST), Nara, Japan

The phytohormone auxin is transported by polar auxin transport system from cell to cell, leading to the asymmetric auxin distribution in plants. Polar auxin transport is mainly dependent on the activity of auxin efflux carriers, PIN-FORMED (PIN) proteins, localized in the plasma membrane with polarity. Recently, MACCHI-BOU 4/ENHANCER OF PINOID (MAB4/ENP), a NONPHOTOTROPIC HYPOCOTYL 3 (NPH3)-like protein, have been reported to regulate polar auxin transport through the control of sub-cellular localization of PIN proteins. However, the contribution of MAB4/ENP to polar auxin transport is restricted because the *mab4/enp* mutation causes mild defects only in aerial organ formation. This suggests that redundant factors may function in polar auxin transport with MAB4/ENP.

There are 31 members of *NPH3* family in the Arabidopsis genome. Four NPH3 members display higher homology to *MAB4/ENP*, named *MAB4/ENP*-LIKE *(MEL) 1-4*. In order to examine the function of *MEL* genes, we first analyzed expression pattern of *MEL* genes and sub-cellular localization of MEL proteins. *MEL* genes displayed different expression pattern from *MAB4/ENP*, but in part overlapping one with *MAB4/ENP*. In their expression domains, MAB4/ENP and MEL proteins were localized nearby the plasma membrane with polarity, almost identical to that of PIN proteins there. Next, genetic interactions between *MEL* genes and/or *MAB4/ENP* were examined. Both *mel1* and *mel2* mutations enhanced *mab4* mutant phenotypes as *mab4 mel1 mel2* triple mutants developed pin-like inflorescences. This suggests that *MEL1* and *MEL2* function in organ formation redundantly with *MAB4/ENP*. Surprisingly, *mel1 mel2 mel3 mel4* quadruple mutants displayed severe defects in the root gravitropism. These results indicate that *MEL* genes function in various auxin-dependent morphogenesis, not only organ formation but also root gravitropism.

P164 The abaxial-side specific expression of *MIR165/166* clearly marks off the *PHB*-expression domain from the *FIL*-expression domain in Arabidopsis leaf primordia

Kiyoshi Tatematsu1, Keiro Watanabe2, Koichi Toyokura2, Toshiaki Tameshige2, Kiyotaka Okada1,2

1National Institute for Basic Biology, Okazaki, Aichi, Japan, 2Kyoto University, Kyoto, Kyoto, Japan

In plant bifacial leaf, one halve of leaf near to shoot apical meristem (SAM) is called adaxial side (upper side of leaf), and the other far to the SAM is named as abaxial side (lower side of leaf). During leaf development, several growth regulatory factors localize in adaxial- or abaxial-side of the primordia. To establish the development of leaf blade with palisade- and spongy parenchyma, an adaxial-side regulator, *PHABULOSA (PHB),* and an abaxial-side regulator, *FILAMENTOUS FLOWER (FIL)*, have to be precisely expressed in each side, respectively. Previously, we reveled that *FIL* expression is restricted in the abaxial-side by its own promoter function (Watanabe *et al*, 2003). However, it is still unknown whether the *PHB* expression domain is completely separated from the FIL expression ones. To reveal the question, we focused on the expression domains of *PHB, FIL* and *MIR165/166* in rabidopsis leaf primordia. The analysis of promoter-reporter fusion genes indicated that the expression domains

of *PHB* and *FIL* were partially overlapped. In contrast, when GFP fused with *miR165/166* recognition/cleavage site of *PHB* was overexpressed, the GFP signal was clearly separated from the YFP signal driven by *FIL* promoter, indicating that *PHB* function domain was clearly marked off from *FIL* function domain. Then, using laser microdissection technique, total RNA was prepared from the adaxial- or abaxial-side of the primordia, respectively, and the localizations of pre-*miR165/166* were checked by semi-qRT-PCR analysis. Among 9 *MIR165/166* genes, 6 transcripts were found only in abaxial-side. Thus, we proposed that the abaxial-specific expression of *MIR165/166* is one of the factors for precise developments of parenchyma cells along the adaxial-abaxial polarity.

P165 *UNICORN*, a negative regulator of YABBY gene *INNER NO OUTER* in Arabidopsis ovule integument development

Balaji Enugutti, Kay Schneitz

Entwicklungsbiologie der pflanzen, Technische Universitet Munchen, Am Hochanger 4, D 85354 Freising, Germany

The mechanisms regulating cell division and cell layer organization in developing plant organs are fundamental to plant growth. However, these mechanisms are not fully understood and require further elucidation. Another interesting question in biology is how gene expression is regulated spatially and temporally within a tissue and or whole organ, according to developmental and environmental cues. Ovule integument layers serve as a good model system for understanding cell division patterns, cell layer organization and gene regulation. Towards this goal, we present here our analysis of *UNICORN (UCN)*, a gene that is involved in ovule integument development in *Arabidopsis thaliana*. The recessive *ucn-1* mutant shows localized mis-regulation of cell divisions in ovule integuments, resulting in protrusions. Such aberrations in cell division patterns are also observed in the epidermal cells of other floral organs. Here, we provide evidence that *UNICORN* acts to downregulate the expression of *INNER NO OUTER*, the abaxial patterning *YABBY* family gene in ovule integuments.

P166 Seeking a meiotic trigger in Arabidopsis

Liz Alvey, Ian Henderson

University of Cambridge, Cambridge, UK

Meiosis is a specialised cell division that gives rise to the haploid cells required for gamete formation and sexual reproduction. The factors that induce cells to undergo meiosis vary greatly between (and within) eukaryotic species and a meiotic trigger has yet to be defined in plants. Here we propose to use a forward genetic screen to identify sterile mutants in Arabidopsis that fail to enter meiosis at the appropriate time and place during development. Fluorescent protein tags will be used to selectively highlight cells undergoing meiosis and the sterile mutants will be investigated for the presence of meiocytes using confocal microscopy. By selecting mutants that exhibit normal early anther and ovule development, but fail to enter meiotic prophase I, we aim to indentify genes that are required for entry into meiosis in Arabidopsis.

P167 Members of the AP2/ERF family of transcription factors act in vascular development

Ziwei Song, Simon Turner, Peter Etchells

University of Manchester, Manchester, UK

Vascular meristems, known as the cambium and procambium, are made up of undifferentiated cells that generate all vascular tissue in a highly ordered process of organised cell divisions. These ordered divisions, result in displacement of older cells which subsequently differentiate into xylem towards the inside of the stem and phloem towards the outside. The receptor kinase *PHLOEM INTERCALATED WITH XYLEM (PXY)* was the first gene identified as being essential for ordered, coordinated cell divisions in the procambium. Ectopic application or over-expression of the *PXY* ligand CLE41 leads to increases in cambial and procambial cell divisions suggesting that *PXY* signalling, in addition to setting the division plane, also promotes the divisions themselves. However, *pxy* mutants demonstrate relatively subtle defects in vascular cell number. One explanation for this is that a compensatory mechanism is activated in the absence of *PXY* that stimulates vascular cell divisions. To identify components of this putative mechanism, we carried out a microarray experiment and identified several members of the AP2/ERF family of transcription factors which are up-regulated in *pxy* mutants. Functional analysis of these genes clearly demonstrates that they are required for vascular cell divisions as combinatorial mutants generate significantly less vascular tissue than wild-type plants and as such they represent novel factors in plant vascular development.

P168 Gibberillins control fruit pattering in *Arabidopsis thaliana*

Nicolas Arnaud1, Thomas Girin2, Karim Sorefan2, Tom Wood2, Robert Sablowski1, Lars Østergaard2

1Cell and Developmental Biology Department, John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK, 2Crop genetics Department, John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK

Successful reproductive strategies are dependent on efficient seed protection and dispersal systems. Fruits have evolved as such systems. The fruit of *Arabidopsis thaliana* is a silique constituted of two valves fused to a central replum by a specific tissue called valve margin. This structure allows the fruit to open easily leading to the release of the seeds. In the past few years, elegant genetic screens have revealed the identity of key regulators involved in the fruit development in Arabidopsis. REPLUMLESS (RPL) and FRUITFULL (FUL) restrict the expression of valve margin identity genes such as *INDEHISCENT (IND)* and *SHATTERPROOF (SHP1/2)* to the valve margins. In contrast, not much is known about molecular events downstream of the core RPL/FUL/IND. By combining microarray analysis and ChIP experiments using an inducible 35S:IND-GR system, we show here that *GA3oxidase1 (GA3ox1)*, encoding a key enzyme in the gibberellins biosynthetic pathway, is a direct target of IND. Expression analysis indicates that *GA3ox1* is

expressed in valve margins. In addition, a mutant defective in GA biosynthesis and transgenic lines with reduced level of GA in the valve margins show impaired valve margin patterning and altered fruit dehiscence. Altogether, these results strongly suggest that GAs are necessary for correct valve margin formation. This work contributes to the overall understanding of molecular events that occur downstream of a key regulator like IND, leading to the development of the sophisticated fruit structure.

P169 Modelling dynamic growth maps of leaf development

Erika Kuchen, Samantha Fox
John Innes Centre, Norwich, UK

To understand how genes control leaf shape and size we are characterising the dynamics of leaf growth in Arabidopsis. We first established a robust staging sytem that can be used to accurately determine the developmental phase of an Arabidopsis leaf. Time-lapse imaging was then used to capture leaf growth during each phase. Growth parameters were extracted from the resulting images using computational techniques. This approach was complemented by sector analysis in which clones expressing GFP were induced and visualised at a range of stages. The results have informed mechanistic models of leaf growth and allowed the role of genes in leaf growth to be explored.

P170 Characterisation of Arabidopsis cathepsin B involved in programmed cell death

Y Ge1, L Bonneau1, VE Rotary1, A Danon1, B Turk2, P Gallois1
1Faculty of Life Science, the University of Manchester, Manchester UK, 2Department of Biochemistry, J. Stefan Institute, Ljubljana, Slovenia

Programmed cell death (PCD) is a process that is conserved between plants and animals. Some proteases involved in animal PCD have homologues in plants. AtCath B is a homologue of human Cathepsin B in *Arabidopsis thaliana*. We produced multiple knock-out (KO) lines by crossing single T-DNA insertion lines for each of the three genes of the CathB family in Arabidopsis. Double KOs lines demonstrated that CathB are involved in the regulation of PCD in Arabidopsis. We showed in our study that UVC-induced PCD and H_2O_2-induced PCD are both reduced significantly in KO mutants. In addition, seedling PCD induced by the herbicide Methyl viologen was reduced in double KO mutant lines. Enzymatic assays using recombinant AtCathB showed similarities and difference between the Arabidopsis and the human enzyme.

P171 Role of polycomb-group genes in commitment to flowering in Arabidopsis

Ralf Müller1, Daniel Schubert2, Justin Goodrich1
1Institute for Molecular Plant Science, University of Edinburgh UK, 2Institute for Genetics, Heinrich Heine University, Dusseldorf, Germany

In Arabidopsis and many other plants, the induction of flowering by photoperiod is stable, so that plants remain committed to flowering even when removed from inductive conditions. We are testing whether the Polycomb-group (Pc-G) genes are required for this floral commitment as they are known to mediate stable changes in cell fate.

Firstly, we created plants with severely depleted Pc-G activity, both by making transgenic plants with steroid dependent Pc-G activity, and also by making *emf2-10 vrn2-1* double mutants with defects in two partially redundant PcG genes. These plants have flowers which show some features of inflorescences, suggesting that floral identity is impaired. To further test this, we shifted wild-type and mutant plants from inductive to non inductive conditions and we observed a reversion from floral to vegetative development in mutants, suggesting that commitment to flowering was impaired.

In a second, whole genome approach we are identifying which genes are regulated by Pc-G in young floral meristems following floral induction, to test whether these include genes that antagonise floral meristem identity. We and others have shown that the chromatin of genes regulated by the Pc-G is characterised by a specific modification, namely trimethylation of lysine 27 on the histone H3 tail (H3K27me3). We are therefore using chromatin immunoprecipitation (ChIP) to identify genes which gain H3K27me3 following floral induction. For this we use the well established *ap1-1 cal-1 AP1-GR* system which provides a large amount of both undifferentiated inflorescence meristem and also (following steroid induction) synchronised floral tissue.

P172 CAST AWAY, a receptor-like cytoplasmic kinase, regulates floral organ abscission

Christian Burr, Michelle Leslie, Michael Lewis, Stephanie Hasty, Patrick Healy, Sarah Liljegren
University of North Carolina-Chapel Hill, (NC), USA

Abscission is a programmed cell-separation process that allows plants to shed their organs. In Arabidopsis flowers, each outer organ has a set of differentiated abscission zone cells at its base which allows the organ to detach after pollination. Our previous studies have shown that the NEVERSHED (NEV) ARF GAP regulates membrane trafficking and is required for organ abscission (Liljegren *et al*, 2009). To identify additional components of pathways that regulate abscission, we carried out a suppressor screen to identify mutations that restore shedding in *nev* mutant flowers. I have been characterizing a receptor-like cytoplasmic kinase, CAST AWAY (CST), which acts as a negative regulator of abscission. *CST* shows a distinct expression profile in floral abscission zones, the pedicel vasculature, root tips, and the stomata in developing leaves. Mutations in *CST* dominantly rescue abscission in *nev* flowers, and *nev cst* abscission zone cells show increased cell expansion. Interestingly, a similar phenotype has been observed in flowers constitutively expressing IDA, a signaling molecule required for abscission (Stenvik *et al*, 2006). To further investigate CST function, I am analyzing its kinase activity *in vitro* and generating CST-YFP transgenic lines to determine its subcellular localization. We are also exploring the genetic

and potential physical interactions between CST and otherreceptor-like kinases that regulate abscission.

P173 The essential role of VLCFAs for cell plate establishment during cell division in *Arabidopsis thaliana*

Liên Bach1, Louise Michaelson2, Richard Haslam2, Yannick Bellec1, Lionel Gissot1, Jessica Marion1, Jean Pierre Boutin1, Martine Miquel1, Frederique Tellier1, Frederic Domergue3, Jonathan Markham4, Frederic Beaudouin2, Jonathan Napier2, Jean Denis Faure1

1INRA Versailles France, 2Rothamsted Research UK, 3CNRS-Universite Bordeaux 2 France, 4Donald Danforth Plant Science Center, USA

Very-long-chain fatty acids (VLCFAs) are involved in the synthesis of several classes of lipids that are essential for membrane structure and function. VLCFAs are elongated from long chain acyl-CoAs by four enzymes associated in the endoplasmic reticulum (ER) in an elongase protein complex. The acyl-CoA dehydratase involved in the third step in acyl-CoA elongation was recently identified as PASTICCINO2 (PAS2) (Bach *et al* 2008). We demonstrated by Bimolecular Fluorescence Complementation that PAS2 interacts specifically *in vivo* with the fourth enzyme of the complex, the enoyl-CoA reductase CER10 in the ER. The *pas2* mutant is characterized by a general reduction of VLCFAs in seed storage triacylglycerols (TGAs), cuticular waxes, complex sphingolipids and by the accumulation of 3-hydroxy-acyl-CoA intermediates. The *pas2* mutant is also associated with abnormal cell proliferation and altered development suggesting that VLCFA are involved in cell division in plants. We will present data demonstrating that cytokinesis was compromised in *pas2* mutant and was associated with modified endomembrane and plasmamembrane dynamics. Our results highlight, for the first time in plants, the essential role of the VLCFAs in cell division during plant development.

P174 Physical interaction between ABBERANT TESTA SHAPE/KANADI4 and AUXIN RESPONSE FACTOR3/ETTIN mediates integument development in Arabidopsis

Dior Kelley1, Alexandra Arreola2, Charles Gasser1

1University of California, Davis, CA, USA, 2Univeristy of North Carolina, Chapel Hill, NC, USA

ABERRANT TESTA SHAPE/KANADI4 (ATS/KAN4) plays two roles during ovule development: providing boundary maintenance between the inner; and outer integuments and directing laminar growth of the inner integument. While it is clear that ATS plays unique roles during ovule development, this putative transcription factor probably does not act alone. We performed a yeast two-hybrid screen to find potential protein interactors using full length ATS as bait and an Arabidopsis pistil cDNA library as prey. This yeast two-hybrid screen identified several proteins involved in transcription including AUXIN RESPONSE FACTOR3/ETTIN (ARF3/ETT) as interacting proteins. We examined the ATS/KAN4 – ARF3/ETT interaction *in planta* using Bimolecular Fluorescence Complementation (BiFC). A direct interaction between ATS-YFPn and ETT-YFPc in transiently transformed onion epidermal cells confirmed the yeast interaction, suggesting that these proteins form a *bona fide* transcriptional complex *in vivo*. Further support for this hypothesis comes from the overlapping expression patterns of *ATS* and *ETT* during ovule development as determined by *in situ* hybridization. The interaction between ATS/KAN4 and ARF3/ETT may represent a more generic binding of KANADIs with ARFs (specifically ARF3/ETT and ARF4), which has been suggested to occur during leaf development to promote abaxial identity. Although the congenital fusion seen in *ats* ovules has not previously been linked with auxin signaling, the nature of this protein-protein interaction implies that transcription of ATS target genes may be mediated by auxin. We hypothesize that in wild-type ovules such a transcriptional response to auxin is directly facilitated by a physical interaction between ATS/KAN4 and ARF3/ETT, which act in concert to promote integument growth. Mutant analyses of *arf3/ett* in combination with *ats* are being utilized to define the functional relationship between these abaxial factors during ovule development.

P175 Interaction of *INDEHISCENT* and *SPATULA* for the valve margin formation in the Arabidopsis fruit

Thomas Girin1, Karim Sorefan1, Teodora Paicu2, Martin O'Brien2, Tom Wood1, David Smyth2, Lars Østergaard1

1John Innes Centre, Norwich, UK, 2Monash University, Melbourne, Australia

Several transcription factors of the bHLH family are known to be key regulators of the fruit patterning in Arabidopsis, including *INDEHISCENT (IND)* and *SPATULA (SPT)*. We have previously shown that IND activity in the valve margins regulates the localisation of PIN auxin efflux carriers via direct regulation of *PID* and *WAG2* expression. This creates an auxin minimum which triggers the formation of the dehiscence zone of the fruit. Accordingly, *ind* mutants present a lack of valve margin formation and the siliques fail to open at maturity. On the *other* hand, *SPT* is known to regulate the formation of medial tissues of the fruit. Here we show that *SPT* is also required for adequate valve margin formation. In *spt* mutants, valve margin tissues are reduced and the fruits are partially indehiscent. Expression analysis and ChIP experiments using an inducible *35S:IND-GR* construct show that *SPT* is directly induced by *IND*. This is consistent with the overexpression of *pSPT:GUS* in *35S:IND* plants. In addition, yeast 2-hybrid experiments as well as localisation experiments in onion epidermal cells suggest that the two proteins interact with each other. Ectopic expression of *IND* leads to defects in inflorescence and floral architecture, which are mainly related to *SPT* activity as they are strongly reduced in a *spt* background. However, no defect is observed in *35S:SPT* plants, suggesting the phenotype is due to the joint activity of *SPT* and *IND*. Taken together our data suggest that *IND* induces *SPT* expression in the valve margins and that the protein-protein interaction with SPT is needed for IND to pattern the dehiscence zone.

P176 Identifying the location of GAL4-GFP enhancer trap lines using TAIL-PCR in *Arabidopisis thaliana*

Jessica Diaz, MariaElena Zavala
California State University, Northridge, Northridge, USA

Arabidopsis thaliana is an important model organism used in genetic and molecular biology used today. It is ideal to work with because of its fast lifecycle (6 weeks from seed germination to senescence), requires very few nutrients and space, and there is a vast amount of information on the genome. In our lab, we are focusing on root tissue development and growth. We are concentrating on locating the position of GAL4-GFP enhancer trap line sequences in the genome of various transgenic Arabidopsis using TAIL-PCR (Liu, 1995). We have adapted this method to work in various lines of transgenic Arabidopsis. Once we have identified where the GAL4-GFP enhancer trap lines are located in the genome, we will be able to use bioinformatics to find a relationship between the site of the trap line and the tissue specificity of roots.

Supported by NIGMS (#5T34GM008395) and CSUN Provost Fund.

P177 Functional analysis of transcripts repressed by the presence of the female gametophyte in the ovule of *Arabidopsis thaliana*

Alma Armenta-Medina, Jean-Philippe Vielle-Calzada
Laboratory of Reproductive Development and Apomixis, Department of Genetic Engineering and Langebio, CINVESTAV Irapuato, Mexico

The life cycle of land plants alternates between a sporophytic (diploid) and a gametophytic (haploid) phase. Whereas in Bryophytes the gametophytic phase prevails over the sporophyte, in Angiosperms the sporophyte is predominant over both the male and female gametophyte. We are interested in elucidating the genetic basis and molecular mechanisms that control the formation of female gametes in Arabidopsis. After performing a global expression analysis using Massively Parallel Signature Sequencing (MPSS), we identified a collection of 2,786 distinct transcripts that are present in ovules of *sporocyteless/nozzle (spl)* lacking a female gametophyte, but not in ovules of wild-type plants. These transcripts could be either repressed by the developing female gametophyte or by a SPL-dependent pathway during normal ovule development. After confirming the differential expression of at least 10 genes that are active in *spl* ovules, we determined their specific pattern of expression by generating transgenic lines containing a translational fusion of their regulatory region to the reporter gene *uidA* (GUS). While the resulting expression patterns are quite diverse, some lines show GUS expression restricted to integumentary cells in the micropylar region, a result suggesting that the female gametophyte has the potential to exert a repressive control over neighboring regions in the ovule.

We are currently using additional mutants to confirm the expression of these genes in the absence of a female gametophyte, and elucidating their function by characterizing insertional T-DNA lines and determining phenotypical changes caused by their ectopic expression.

Our results point towards the importance of a genetic cross-talk necessary for the formation of the female gametophyte within the sporophytically derived ovule.

P178 Arabidopsis *VEN3* and *VEN6* encode carbamoyl phosphate synthetase subunits

Almudena Mollá-Morales, Raquel Sarmiento-Mañús, Rebeca González-Bayón, Pedro Robles, Víctor Quesada, Matthew Hannah, Lothar Willmitzer, José Manuel Pérez-Pérez, María Rosa Ponce, Jose Luis Micol
1Division de Genetica and Instituto de Bioingenier, Universidad Miguel Hernandez, Elche, Spain, 2Max-Planck-Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

The Arabidopsis *venosa (ven)* mutants exhibit reticulate leaves, whose vascular network can be clearly distinguished as a green reticulation on a paler lamina. To gain insight into the processes through which epidermal, mesophyll and vascular tissues contribute to leaf patterning, we characterized five ven mutants. The four *ven3* alleles and the single *ven6* allele studied were semidominant and caused the primary and secondary veins to look much greener than the interveinal lamina regions. A metabolomic analysis revealed that the *ven3* and *ven6* mutants accumulate ornithine.

The *VEN3* and *VEN6* genes were positionally cloned and found to encode the two subunits of the Arabidopsis carbamoyl phosphate synthetase.

P179 The expression pattern of *AGP18* reveals coordinated sporophytic and gametophytic control during ovule development in Arabidopsis

Edgar Demesa-Arévalo, Jean-Philippe Vielle-Calzada
Laboratory of Reproductive Development and Apomixis, Department of Genetic Engineering and Langebio, CINVESTAV Irapuato, Mexico

Our current understanding of cell differentiation in flowering plants involves 2 developmental steps: determination - generally caused by the asymmetric inheritance of cytoplasmic determinants or through cell-to-cell communication, and cell fate specification. The establishment of a reproductive lineage is known to be tightly coordinated, both temporally and spatially. Classical arabinogalactan proteins (AGPs) are a group of GPI-anchored signaling proteins that adopt highly specific patterns of distribution in vegetative as well reproductive organs. *AGP18* is a gene essential to initiate female gametogenesis in Arabidopsis. Although *AGP18* is expressed in cells that spatially and temporally define the sporophytic to gametophytic transition in the ovule, the regulation of its activity remains elusive. The presence of several *cis*-acting regulatory elements suggests that *AGP18* is regulated

by hormonal and epigenetic mechanisms. To characterize in detail the pattern of expression and localization of *AGP18*, we dissected its promoter activity by designing 5 distinct transcriptional fusions that include different portions of its regulatory region. Reporter expression initiated at the onset of female gametogenesis and was confined to a ring of integumentary and nucellar cells surrounding the female gametophyte; expression was also detected in the fully differentiated female gametophyte. The general comparison of mRNA and protein activity suggests that *AGP18* could act in a non-cell autonomous developmental pathway.

P180 Characterization of a gene homologous to At1G74730 *(BnMicEmU)* upregulated in embrogenic *Brassica napus* microspore cultures

Fariba Shahmir, Peter Pauls
University of Guelph, Guelph, ON, Canada

A mild heat stress of *Brassica napus* microspore cultures causes some of the cells to switch their developmental pathway from pollen maturation to embryo formation. Embryo formation in microspore cultures closely resembles zygotic embryo development in morphology, protein synthesis and physiology. In a microarray screen of ESTs from sorted embryogenic versus nonembryogenic cells 120 transcripts that were upregulated more than 2-fold in the embryogenic cells were identified, including a sequence that was homologous to the Arabidopsis gene At1G73740. The function of this gene is unknown. The objectives of the current work were to characterize the complete gene and investigate the function of the gene by gene silencing and overexpression studies in Arabidopsis and *B. napus.* The complete gene was isolated and sequenced from *B. napus* by PCR and RT-PCR using primers designed from the At1G73740 sequence and named as *BnMicEmUP* (*B. napus* microspore embryogenesis up regulated gene). Three distinctive *BnMicEmUP* genes with nucleic acid similarities ranging from 82 to 83% to Arabidopsis At1G73740 were isolated from *B. napus* cv Topas. Real-time PCR showed that expression of one of these genes is expressed in induced (embryogenic) microspore cultures but not in noninduced cultures. In contrast, another form of *BnMicEmUP* is expressed constantly in induced and noninduced cultures and is also expressed in leaf, root, stem and pollen tissue. To further investigate the role of *BnMicEmUP* in embryogenesis, two silencing constructs were made with the Pfgc5941vector and vectors with a 35spromoter (pBI121) and an inducible promoter (pER8) were constructed for over-expression of the *BnMicEmUP* gene. These constructs were delivered to Arabidopsis by Agrobacterium using the floral dip method. Their effects on gene expression and embryo formation are currently being investigated. The information from studies of microspore embryogenesis is immediately applicable to improving this system for producing homozygous plants for plant breeding. In addition, the basic information on embryogenic processes leads to a greater understanding of totipotency in plants.

P181 The role of the trehalose-6-phosphate sugar signal in coordinating leaf development to plant sugar status in *Arabidopsis thaliana*

Alexander Ivakov, John Lunn, Vanessa Wahl, Mark Stitt
Max Planck Institute for Molecular Plant Physiology, Potsdam, Germany

Trehalose-6-phosphate (Tre6P) is emerging as a major signal metabolite responding strongly and specifically to sucrose availability in the plant and regulating starch metabolism downstream. Previous work has shown that constitutive modulation of Tre6P levels in plants leads to strong developmental and growth phenotypes affecting both reproductive as well as vegetative development, as well as being one of the only examples of genetic modification of leaf photosynthetic capacity. This has often been interpreted as a pleiotropic effect to phenotypes related to starch metabolism. In this study we demonstrate that modulation of Tre6P levels in *Arabidopsis thaliana* through over-exression of bacterial enzymes synthesising or degrading Tre6P affects cell division and cell expansion processes in leaves proportionally to the levels of Tre6P in the plant, resulting in large changes in leaf size and growth rate as well as affecting leaf shape, photosynthetic capacity and specific leaf area. We compare these changes to the developmental responses of leaves to high and low light, a manipulation of plant sucrose status. In addition, we employ the starchless *pgm* mutant to demonstrate that these effects are independent of starch, as previously thought. These results suggest that Tre6P may be an important signal transducing plant sucrose availability into leaf developmental responses and could be involved in adaptive growth responses resulting in optimisation of leaf functional properties in response to environmental conditions.

P182 GPCR-type G proteins are essential for normal growth and development in Arabidopsis

Felix W Jaffé, Gian-Enrico C Freschet, Billy M Valdes, Jonathon G Jerram, Matthew J Terry, Lorraine E Williams
School of Biological Sciences, University of Southampton, SO16 7PX

GTG1 and GTG2 are membrane proteins with 8-9 predicted transmembrane domains that are highly conserved in plants, animals, and fungi. They have recently been classified as G protein-coupled receptor-type G proteins that function as ABA receptors in Arabidopsis. Double mutants in a Ws background were reported to show ABA hyposensitive phenotypes (Pandey *et al*, 2009, Cell 136: 138-148).

Here we report on additional phenotypic characteristics observed in independently isolated *gtg1gtg2* double mutants in Col and Ws backgrounds with new alleles compared to the previously isolated double mutant. In seedlings and immature plants, these new *gtg1gtg2* double mutants, but not the single mutants, exhibit altered root and shoot phenotypes in comparison to wild type under a variety of conditions. In the mature plant, fertility-related defects were observed. Analysis using both *pGTG::GUS* and *pGTG::GTG-GUS* fusions showed that

showed that *GTG1* and *GTG2* have very similar expression patterns and are present throughout the plant. It is likely that *GTG1* and *GTG2* have resulted from a relatively recent gene duplication event. This is supported by DNA and protein sequence conservation (91% at the amino acid level), similarity of expression localisation, the presence of single copies in other plant species and their apparent functional redundancy. Microarray analysis has been used to compare wild type gene expression to that of and double mutant in order to help establish the function of *GTG1* and *GTG2*. We have identified expression differences in genes important in cell wall function, amino acid metabolism and hormone response. Current work is focussed on determining whether the differences in growth characteristics and gene expression that we have observed are dependent on the proposed role of GTGs as ABA receptors or whether these proteins have additional functions.

P183 Expression analysis of the snf1-like AKIN10 kinase and domains of interaction with the transcription factor PTL

Martin O'Brien, D R Smyth

Monash University, Victoria 3800, Australia

We are characterizing the role of the PETAL LOSS (PTL) trihelix transcription factor and its role in perianth development in *Arabidopsis thaliana*. There is evidence that the PTL protein is involved in growth suppression between emerging sepals, leading to their separation and allowing sufficient space for petals to emerge internal to the inter-sepal zone. To identify proteins that interact with PTL, we conducted a yeast two-hybrid screen and identified a SNF1-related protein kinase AKIN10 was isolated. AKIN10 and PTL interaction was proved *in vitro* by pull-down assays and through the use of bimolecular fluorescence complementation (BiFC), we were also able to show this interaction *in planta*. We also showed that the non-kinase domain of AKIN10 is sufficient to bind PTL through its N-terminus where PTL contains a serine/threonine rich region that could be subject to phosphorylation. In this regard, *in vitro* phosphorylation assays by AKIN10 were not conclusive. Promoter analysis of *AKIN10* has been undertaken by fusions to GUS, and PTL expression is included in the *AKIN10* transcription domain. Furthermore, important regulatory elements of the *AKIN10* gene are situated in the transcribed region of *AKIN10* and not in the promoter *per se*. We have also over-expressed the kinase in the region defined by *PTL* expression to see if this will affect in any way the regulation of PTL We are presently trying to understand the biological relevance of this interaction considering that *AKIN10* has been shown to sense energy deprivation and target a range of transcription factors involved in metabolism.

P184 Disruption of stem cell regulation by ectopic GIK activity in *Arabidopsis thaliana*

Kian Hong Ng, Toshiro Ito

Temasek Life Sciences Laboratory, Singapore

For decades, we have learned about the powerful "self-regeneration" of plant species that far exceeds their animal counterparts. It is hence an enticing task to delineate the mechanism underneath the remarkable regeneration capacity of plant. Experiments concerning floral bisection and laser ablation of shoot apical meristem have pioneered the field with alluring clues to be trailed (Hicks and Sussex, 1971; Reinhardt *et al*, 2003). The canonical WUSCHEL (WUS)-CLAVATA (CLV) pathway in *Arabidopsis thaliana* is largely responsible for the maintenance and differentiation of the stem cells both in inflorescence and floral meristem (Laux *et al*, 1996; Mayer *et al*, 1998). Defective WUS function would lead to precocious termination of meristem activity. We show that by ectopically expressing a nuclear matrix region binding protein, GIANT KILLER (GIK), we are able to induce the plant into a state of ectopic meristem formation. This intrinsic regeneration of new stem cell pool is closely associated with the level of WUS in the meristem region, as we found that GIK-mediated transcriptional repression through epigenetic changes of WUS genome is precedent to the ectopic regeneration of new stem cell centers. We propose that suboptimal WUS level in the meristem vicinity serves as a sensing mechanism for the plant to reinstate regeneration signaling that lead to formation of new stem cell centers. We will present the molecular model on stem cell maintenance and regeneration in plants.

P185 The role of AGL63 in fruit growth and development and its function in concert with ABS/TT16

Robert Erdmann

University of Bremen, Evolutionary Developmental Genetics Group, Germany

B class floral homeotic genes confer stamen and petal identity of angiosperms flowers. The sister clade of the B genes termed *Bsister (BS)* genes and interestingly their members are mainly transcribed in female reproductive organs (ovules and carpels) and are conserved throughout seed plant evolution. The *Arabidopsis Bsister (ABS*) gene knock-out mutants show an altered seed pigmentation and endothelium malformation. This comparatively mild phenotype of the *abs* mutant led to the hypothesis that other genes might act redundantly to *ABS*. One such candidate gene is *AGL63*, which appears to be a truncated paralog of *ABS* lacking the C-terminal domain and a part of the K domain but shows high sequence similarity to *ABS* in the remaining protein parts. qPCR expression analysis of *AGL63* revealed predominant expression in reproductive part whereas *ABS* is expressed exclusively in buds, flowers and siliques. Plants constitutively over expressing *AGL63* exhibit an early flowering phenotype and an altered inflorescence structure. Additionally, homeotic conversions of sepals into gynoecium-like structures occur, the petals are lost completely in a large fraction of the plants, and disintegrated whorl structure was observed. Protein-protein interaction between ABS and AGL63 could be shown by EMSA and Yeast-Two Hybrid analyses. Surprisingly, AGL63 does not form heterodimers with SEPALLATA3 (SEP3), which is known to be a common partner for floral transcription factors. Promotor:GUS constructs exhibit expression of *AGL63* in the carpel wall. We are currently investigating four mutant

lines of *agl63* which seem to deviate in their cell size in fruits when compared to the wt suggesting an important role for AGL63 in fruit growth and development.

P186 PINs or no PINs, that is the question: PIN1-independent organ initiation

Bernadette Guenot, Cris Kuhlemeier
University of Berne, Switzerland

Lateral organs of plants, like leaves and flowers, are initiated at the shoot apical meristem (SAM), a dome-shaped stem cell niche at the tip of stems. The current molecular models postulate that the growth-promoting hormone auxin accumulates at sites of future organ outgrowth in the SAM. PIN1, an auxin efflux carrier, plays a major role in the mechanisms leading to the creation of auxin gradients in the SAM. Indeed, *pin1* mutants display a striking phenotype of naked, pin-shaped inflorescences lacking lateral organs. However, although experimental evidence and mathematical modelling of PIN1-based processes account for the mutant phenotype of *pin1* inflorescence stages, they don't take into consideration its vegetative phenotype – for *pin1* plants still produce a number of functional, though misshapen, leaves. An obvious explanation for this phenomenon could be that other members of the PIN family of auxin efflux carriers act redundantly in the vegetative SAM. Surprisingly, none of the analysed PINs was found in the SAM, although several of them were expressed in the epidermis and sometimes vasculature of leaves, suggesting a role in leaf development. Also, some of them, namely PIN2 and PIN3, were found in the epidermis soon after organ initiation. This suggests that they may deplete surrounding meristematic areas of auxin, therefore leading to the formation of auxin gradients that are sufficient for organ initiation. The aim of this study is to determine whether other members of the PIN family are indeed responsible for leaf initiation in the absence of PIN1.

P187 *TRUMPET LEAVES* interacts with *ASYMMETRIC LEAVES1* in specifying leaf dorso-ventral polarity

Alexis Moschopoulos, Mary Byrne
John Innes Centre, Norwich, UK

Leaf initiation and the establishment of leaf dorso-ventral polarity provides a good model system for studying both fundamental genetic and molecular mechanisms of cell differentiation and, more specifically, the events controlling the proper development of the plant leaf. In order to better understand these processes, a mutagenesis screen was carried out on an Arabidopsis line carrying a lesion in the *Asymmetric leaves1 (AS1)* gene, a transcription factor essential for proper leaf development. A modifying mutation identified in this screen that causes the production of many radial and trumpet-shaped leaves in the *as1* background was isolated, and named *trumpet leaves (tlv)*. Mutants defective in specifying dorsal leaf fate produce leaf-like organs that are either ventrally-radial or trumpet-shaped, suggesting that this gene acts to specify dorsal cell fate. Genetic mapping suggests the *TLV* gene may be one previously unknown to function in leaf development. The single *tlv* mutant has mild leaf shape and dorso-ventral patterning defects, while in the *as1* background it is severely compromised in the process of leaf patterning. Phenotypic and gene expression analysis to more accurately determine the patterning defects is being carried out. Further characterisation of this allele, in combination with other alleles known to affect dorso-ventral patterning, may provide novel insights into this fundamental process of plant development.

P188 Analysis of the DUO1 regulatory network controlling male germline development

Michael Borg, Lynette Brownfield, Said Hafidh, Anna Sidorova, David Twell
Dept of Biology, University of Leicester, UK

In angiosperms, the male gametophyte plays a vital role in plant fertility through the generation and delivery of two sperm cells to the embryo sac for double fertilisation. Despite its importance, the mechanism integrating germ cell proliferation and specification during gametogenesis has remained elusive. Our recent isolation of *duo pollen (duo)* mutants, which specifically block germ cell division, has provided compelling evidence for gametophytic regulation of germ line cell cycle progression.[1] In the *duo1* mutant, the germ cell fails at the G2/M boundary of the cell cycle to produce pollen with a single germ cell unable to fertilise. *DUO1* encodes a novel R2R3 MYB transcription factor that is specifically expressed in the male germline.[2] Our recent findings have demonstrated that *DUO1* regulates the expression of key genes required for fertilisation as well as regulating germ cell expression of the G2/M regulator CycB1;1.[3] We further showed that male germline-restricted expression of *DUO1* does not depend on a proposed de-repression mechanism and involves positive promoter elements. Thus, these findings demonstrate that *DUO1* is the key regulator in the production of functional sperm cells that has a novel integrative role linking germ cell specification and cell cycle progression.[3] We have recently described an approach whereby we ectopically expressed *DUO1* in seedlings under control of an inducible promoter in order to demonstrate *DUO1*-dependent expression of male germline genes.[3] Here we describe how we have exploited this system to identify more than 50 putative *DUO1* targets by microarray analysis. A significant number of these genes show DUO1-dependence in transient luciferase assays and are currently being verified as genuine *DUO1* targets *in planta.* Mechanistic data concerning DUO1 target activation and its DNA binding sites will also be presented. Together this data provides compelling insights into the scale and architecture of the *DUO1* regulatory network controlling the production of functional sperm cells.

1 Durbarry *et al* 2005 PlantPhys 137:297-307
2 Rotman *et al* 2005 CurrBio 15(3):244-248 3 Brownfield et al 2009 PLoSGenet 5(3):e1000430

P189 Understanding the role of farnesyl pyrophosphate synthase in terpenoids metabolism using *Arabidopsis thaliana* mutants

Pui-Man Yu1, Qing Zhang1, Dongtao Ren2, Dianjing Guo1

1The Chinese University of Hong Kong, Hong Kong SAR, China, 2China Agricultural University, Beijing, China

Terpenoid is the largest class of natural products essential for both primary and secondary metabolism of the plants. Two distinctive terpenoids biosynthetic pathways exist in higher plants, namely, the cytosolic Mevalonate (MVA) and the plastidic Methylerythritol 4-phosphate (MEP) pathway. Although located in separate compartments, it has been suggested that cross talk exists between these two routes. Farnesyl diphosphate synthase (FPS) is one of the key enzymes situated at the branching point of multiple pathways leading to production of sesquiterpene, triterpenes, polyterpene, and sterols. In Arabidopsis, two FPSs (*FPS1* and *FPS2*) have been identified and cloned. Although their temporal and spatial expression pattern has been well characterized, the exact functional roles of these two enzymes are still largely unknown.

In the present work, we aim to investigate the roles of the two FPSs in controlling the metabolic flux of terpenoid biosynthesis using systematic approach. Transcriptome and metabolic profiles of Arabidopsis *fps1* and *fps2* mutants were measured using Affymetric GeneChip and GC-MS techniques. The results showed that perturbation of FPSs had only limited effect on the transcriptome, with slightly higher impact on genes participate in the MEP pathway than those in the MVA pathway. The differentially expressed genes were grouped according to GO terms under the category of molecular function. Genes encoding enzymes involved in stress and defense response, e.g. *GRX* and *TRX*, were altered in both *fps* mutants.

The fact that *fps1* and *fps2* mutants exhibit different gene expression profiles indicates the distinctive roles of these two enzymes may plant in terpenoids biosynthesis. The non-correlation between transcriptome and metabolome suggest a possible post transcriptional regulatory mechanism in terpenoid metabolism.

P190 Identification of Arabidopsis potassium deficient signaling components via full-length cDNA over-expressor (FOX) gene hunting system and AtHAK5

Celine Diaz1, Min Jung Kim2, Daniel Schachtman2, Ryoung Shin1

1RIKEN Plant Science Center, Yokohama, Japan, 2Donald Danforth Plant Science Center, St. Louis, MO, USA

AtHAK5 encodes a high affinity potassium transporter and is the only gene in the *AtKT/HAK/KUP* family that is up-regulated by potassium deprivation and rapidly down-regulated with resupply of potassium.[1] To identify the signaling molecules upstream of AtHAK5 and those that may be more generally involved in response to potassium starvation, we created the Arabidopsis containing a potassium starvation inducible *AtHAK5* promoter::luciferase. The higher luminescent of *AtHAK5*promoter::luciferase plants under potassium starved condition was confirmed compared to the full nutrient condition. Dr. Matsui's group in RIKEN, Plant Science Center have developed a gain-of function system that named FOX hunting system (Full length OvereXpress gene hunting system).[2] The Fox hunting system constitutes of about 10,000 independent full length Arabidopsis cDNAs under 35S constitutive promoters. The FOX libraries were transformed into *AtHAK5* promoter::luciferase plants. To find the components of potassium deficient signaling, we identified lines that showed higher luminescence under full nutrient conditions. More than 500 candidate lines have been identified and their functional analyses are ongoing.

1 Ahn SJ, Shin R, Schachtman DP (2004) Plant Physiol. 134:1135-1145.
2 Ichikawa T, Nakazawa M, Kawashima M *et al*, (2006) Plant J. 48:974-985.

P191 Functional analyses of phosphorylation sites in the activation loop of Arabidopsis phototropin2

Shin-ichiro Inoue, Yuta Tomokiyo, Ken-ichiro Shimazaki

Department of Biology, Faculty of Science, Kyushu University, Fukuoka, Japan

Phototropins (*phot1* and *phot2*) are blue light-receptor protein kinases, and mediate the blue light responses such as phototropism, chloroplast accumulation response, leaf flattening, leaf positioning, and stomatal opening in *Arabidopsis thaliana*. *Phot2* specifically induces the chloroplast avoidance response in response to strong blue light. The Ser residues of the kinase activation loop in *phot1* are autophosphorylated by blue light and the phosphorylation is required for the *phot1*-mediated responses mentioned above. However, the functional role of the autophosphorylation in *phot2* for the *phot2*-mediated responses has not been understood. In this study, we substituted the conserved residues of Ser-761 and Ser-763 with Ala (S761A S763A) in the activation loop, and analyzed their functions by investigating the *phot2*-mediated responses after the transformation of the phot1 *phot2* double mutant with this mutant *phot2* construct. The transgenic plants expressing mutant *phot2* exhibited the inhibited responses and those expressing *phot2* with S761D S763D mutations showed the normal responses. The substitution of both Ser-761 and Ser-763 with Ala in *phot2* did not significantly affect the kinase activity *in planta*. From these results, we conclude that phosphorylation of the Ser-761 and Ser-763 in *phot2* is a primary step that mediates the signaling between photochemical reaction and physiological events.

P192 Root developmental responses to heterogeneous water and nitrogen supply

Nick Chapman1,2, Tony Miller1, Richard Whalley1, Keith Lindsey2

1Rothamsted Research, Harpenden, Herts, AL5 2JQ, UK, 2School of Biological and Biomedical Sciences, University of Durham, South Road, Durham, DH1 3LE, UK

The combined influence of water and nitrogen on plant root morphology and development is poorly understood. In temperate soils, mobile nitrate anions dissolved in the soil water are delivered to the root by water moving in fluxes through the soil generated by transpiration. Root architecture exhibits developmental plasticity and roots can grow to exploit local high concentrations of nitrogen which they encounter as they move through the soil.

Root development and growth is in part regulated by the plant hormones auxin and ethylene. Changes in the tissue concentrations and transport pattern of these hormones can modify the growth of primary roots, the development of laterals and the production of root hairs. This information has been obtained using specific chemical treatments and *Arabidopsis thaliana* (L.) mutants grown on agar-filled Petri dishes, often containing high concentrations of nutrient solutions, and frequently supplemented with sucrose. In the soil, roots encounter a heterogeneous environment that is very different from the surface of nutrient rich agar. So, can we trust extrapolation from lab-based culture to natural conditions? The development of a novel experimental system, based on sand culture, enables the flux of water and nitrogen to the root to be manipulated in different parts of the root system. This facilitates the realisation of differences in water potential and hydraulic conductivity, and hence the flux of water and dissolved nitrate to the root, bridging the gap between the lab and the field.

A. thaliana seedlings (Ws) grown on agar and sand appear to show a difference when compared for root morphology characteristics. Within the system, it is also possible to visualise *in situ* Green Fluorescent Protein fusions, providing an attractive tool for visualising localized protein responses to nutriment regimes.

P193 PRR7 and the regulation of the circadian clock in Arabidopsis

Linsey Newton1, Steve Kay2, Eva Farre1

1Michigan State University, East Lansing, MI, USA, 2University California San Diego, La Jolla, CA, USA

Transcriptional and translational feedback loops regulate circadian clocks in eukaryotes. In Arabidopsis the morning expressed MYB transcription factors *CIRCADIAN CLOCK ASSOCIATED (CCA1)* and *LATE ELONGATED HYPOCOTYL (LHY)* form a transcriptional feedback loop with the evening expressed pseudo-response regulator *TIMING OF CHLOROPHYLL A/B BINDING PROTEIN (TOC1/PRR1). TOC1* is a member of a small gene family of circadian regulated *PRRs*. We had shown that the morning expressed *PRR7* and *PRR9* form an additional loop with *CCA1* and *LHY*, indicating that interlocked feedback loops regulate the plant circadian clock as has been shown to occur in animals. Recent results indicate that all PRRs are posttranslationally regulated. We showed that PRR7 is phosphorylated in a circadian regulated manner. Furthermore its levels are regulated by both circadian and light dependent mechanisms.

P194 Constitutive expression of basal disease resistance in the Arabidopsis ecotype C24 does not impair abiotic stress tolerance or plant fitness

Ulrike Bechtold1, Rhonda Meyer2, Jurriaan Ton3, Thomas Altmann2, Hideki Takahashi4, Philip Mullineaux1

1Univeristy of Essex, Colchester, UK, 2Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany, 3Rothamsted Research, Harpenden, UK, 4Tohoku University, Sendai 981-8555, Japan

For plants, flexibility in responding to multiple challenges is crucial if the plant is to show fitness in different environmental conditions. We analysed 5 accessions for drought resistance, water productivity and biotic stress tolerance. C24 contains the dominant *RCY1* gene conferring resistance to cucumber mosaic virus, which is allelic to *RPP8* in Ler. In C24, RCY1 is considered to be the major contributor to a range of biotic resistances. We screened *rcy1* mutants and a RIL population (C24 x Col-0, IPK Gatersleben) for virulent Pseudomonas syringae and *Hyaloperonospora arabidopsidis* resistance, respectively. Results show that there are multiple loci involved in pathogen resistance. Interestingly, a large proportion of the biotic stress resistance phenotype observed in C24 is similar to *constitutive expressor of PR1* mutants (*cpr*). Microarray analysis of C24 vs. Col-0 showed that a high proportion of stress- and salicylic acid (SA)-dependent genes are up regulated in C24. This coincides with increases in SA, hydrogen peroxide and glutathione. A comparison of up-regulated genes in C24 with *cpr5-1* microarray data found a significant overlap between the two datasets, emphasising the similarities in the observed biotic resistance phenotypes.

C24 also shows an *RCY1* independent resistance to drought stress, and a much improved water productivity in comparison to other accessions. This corresponds with a shift from vegetative growth in favour of reproductive growth, yielding an improved biomass water ratio (BWR). While transpiration is reduced, crucially, photosynthetic electron transport and carbon fixation are maintained in C24. In *cpr* mutants, BWR and total seed yield are much reduced, supporting the notion that constitutive pathogen resistance normally has negative effects on plant fitness.

P195 The Arabidopsis SUCROSE TRANSPORTER 2 (SUC2) regulates plant responses to phosphate starvation, ion homeostasis, and root development

Mingguang Lei1, Yidan Liu1, Kashchandra Raghothama2, Dong Liu1

1Department of Biological Sciences and Biotechnology, Tsinghua University, Beijing 100084, P.R.China, 2Department of Horticulture and Landscape, Purdue University, West Lafayette, IN 47907, U.S.A.

When plants are under phosphate (Pi) starvation, they exhibit an array of developmental, biological, and

physiological responses to cope with the stress. It has also been known that sugar plays a critical role in mediating plant responses to Pi starvation. However, the molecular mechanism underlying these responses is largely unknown. To dissect the signaling pathway associated with Pi starvation, we isolated an Arabidopsis mutant, *hps1* (*hypersensitive to phosphate starvation 1*), which shows enhanced expression of Pi starvation-induced genes, and overaccumulation of anthocyanin and starch under low Pi condition. The homeostasis of several nutrients, such as Pi, Fe, Zn, etc, was also perturbed in the *hps1* plants. In addition, the *hps1* plants exhibit a dramatic change in root architecture, in term of primary root growth, root hair and lateral root formation. Under Pi starvation, the root meristem size and activity in *hsp1* plants was much more reduced, and on the day 11 after germination, almost all the root meristematic cells were dedifferentiated. Further analysis indicated that the mutant phenotype is caused by ectopic overexpression of *SUCROSE TRANSPORTER 2 (SUC2)* gene. As a consequence, the mutant accumulates more sucrose in both its roots and shoots. Furthermore, when the *SUC2* gene was overexpressed in the WT plants under the control of CaMV 35S promoter, it can recapitulate the phenotype of *hps1* plant. Our results demonstrated that *SUC2* gene plays an important role in regulation of plant responses to phosphate starvation, ion homeostasis, and root development. Finally, a genome-wide comparative analysis of the gene expression patterns between WT and *hps1* plants is in progress. The results of these studies will be presented and discussed.

P196 Sumoylation of ABI5 by the Arabidopsis SUMO E3 ligase SIZ1 negatively regulates abscisic acid signaling

Kenji Miura1,2, Jiyoung Lee3, Jing Bo Jin2, Chan Yul Yoo2, Tomoko Miura2, Paul M Hasegawa2

1University of Tsukuba, Tsukuba, Japan, 2Purdue University, West Lafayette, IN, USA, 3Geongsang National University, Jinju, Korea

SUMO (small ubiquitin-related modifier) conjugation (sumoylation) to protein substrates is a reversible post-translational modification that regulates signaling by modulating transcription factor activity. Here, we presents evidence that the SUMO E3 ligase SIZ1 negatively regulates abscisic acid (ABA) signaling that is dependent on the bZIP transcription factor ABI5. Loss-of-function, T-DNA insertion *siz1-2* and *siz1-3* mutations caused ABA hypersensitivity for seed germination arrest and seedling primary root growth inhibition. Further, expression of genes that are ABA-responsive through ABI5-dependent signaling (e.g. *RD29A*, *Rd29B, AtEm6, RAB18* and *ADH1*) was hyper-induced by the hormone in *siz1* seedlings. *abi5-4* suppressed ABA hypersensitivity caused by *siz1* (*siz1-2 abi5-4*), revealing an epistatic genetic interaction between *SIZ1* and *ABI5*. A K391R substitution in ABI5 [ABI5(K391R)] blocked SIZ1-mediated sumoylation of the transcription factor *in vitro* and in Arabidopsis protoplasts indicating that ABI5 is sumoylated through SIZ1 and that K391 is the principal site for SUMO conjugation. ABI5(K391R) expression in *abi5-4* plants caused greater ABA hypersensitivity (gene expression, seed germination arrest and primary root growth inhibition) than ABI5 expression in *abi5-4*. Together, these results establish that SIZ1-dependent sumoylation of ABI5 attenuates ABA signaling. The double mutant *siz1-2 afp-1* exhibited even greater ABA sensitivity than the single mutant *siz1*, suggesting that SIZ1 represses ABI5 signaling function independent of AFP1.

P197 Identifying and characterizing key components of CO_2 signal transduction in Arabidopsis

Yun-Kuan Liang1, Cecilia Tagliavia2, Alistair Hetherington1

1School of Biological Sciences, University of Bristol, Woodland Road, Bristol, BS8 1UG, UK, 2Department of Biological Sciences, University of Lancaster, Lancaster, LA1 4YQ, UK

Stomata are tiny pores surrounded by guard cells, which are responsible for regulating the size of the openings and therefore the gas exchange. Air containing carbon dioxide (CO_2) enters the plant through these pores where it is used in photosynthesis and respiration, whereas water vapour is released into the atmosphere through these pores in transpiration. High CO_2 concentrations repress stomatal opening, while reversely low concentrations promote opening. In this study, we tried to understand how plants may respond to the escalating increasing concentration of atmospheric CO_2. We used infrared thermal imaging to isolate mutants, which showed aberrant leaf warming when subjected to elevated CO_2 levels than atmospheric CO_2. A couple of candidates which are less responsive to high [CO_2] but have different alterations to applied ABA, darkness and calcium had been selected for map-based gene cloning and further investigations. We will provide evidence how the mutations might affect the CO_2 signal transduction and the stomatal development as well.

P198 Siamese-related proteins: Novel plant-specific cell cycle inhibitors induced by stress

Claire Lessa Alvim Kamei, Hilde Van den Daele, Toon Cools, Lieven De Veylder

VIB Department of Plant Systems Biology, Ghent University, B - 9052 Gent, Belgium

The plant cell cycle is a fundamental biological process due to its importance to plant growth and development. Success relies on events well coordinated by protein degradation, mitogen-induction of D-type cyclins, and association with CDK inhibitory proteins, such as ICK/KRP and SIAMESE. Based on sequence analysis, novel *SIAMESE* - related genes were found in Arabidopsis, yielding to a family comprising 13 members. Microarray and quantitative expression analysis on plants exposed to different genotoxic stresses showed an up-regulation of *SMR4, SMR5* and *SMR7*. Cell cycle inhibitory activity of these 3 *SMRs* was illustrated by overexpression analysis, yielding hemi-sterile plants with short stature and a serrated leaf phenotype. The interaction of SMR4, SMR5

and SMR7 with CDKA;1 was demonstrated by Tandem Affinity Purification. The expression pattern was mostly concentrated on the root tip, and as expected showed to be strongly induced upon the exposure to DNA stress inducing drugs, such as bleomycin and hydroxyurea. Further analyses have been conducted in order to address more precisely the role of those genes responding to stress. Our data now suggests an interconnection between these novel cell cycle inhibitors and the resistance to genotoxic stress.

P199 Similarities and differences between STO and its homologous protein STH

Marta Rodriguez-Franco, Dominic Jutt, Katrin Marquardt, Felipe Sarmiento, Eija Schulze, Huili Yan, Gunther Neuhaus

University of Freiburg, Freiburg, Germany

Salt Tolerance (STO) and its homologue STH, are two members of the double B-box Zn finger family of proteins in *Arabidopsis thaliana*. Eight members of this family have been identified in Arabidopsis (Kumagai *et al* 2008; Chang *et al*, 2008). From those, two members (STH2 and LZF1/DBB3) have been characterised as positive regulators of photomorphogenesis (Datta *et al*, 2007; Chang *et al*, 2008; Kumagai *et al*, 2008) whereas four (STO, STH, DBB1a and DBB1b) are characterised as negative regulators of light signalling during de-etiolation (Indorf *et al*, 2007; Khanna, *et al*, 2006; Kumagai *et al*, 2008).

In this work we will present results comparing the regulation of the expression, subcellular localization, and molecular characterisation indicating that STO and STH despite its high similarity present some differences in their mode of action.

P200 Calcium regulated transcription in response to biotic and abiotic stresses

Hillel Fromm, Yael Galon, Orly Snir, Aliza Finkler

Tel Aviv University, Tel Aviv, Israel

In response to environmental stimuli, including abiotic (cold, heat, salt, drought, light, touch) and biotic stresses, Ca^{2+} concentrations are transiently elevated and Ca^{2+} transients are transduced by various types of Ca^{2+}-binding proteins. However, little is known about the mechanisms mediating Ca^{2+}-responsive gene expression in plants. Transcriptome analysis of plants shortly after induction of cytosolic Ca^{2+} transients revealed the identity of ABREs (Abscisic Acid Responsive Elements) as Ca^{2+}-responsive *cis*-regulatory elements.[1,2] Furthermore, analysis of a family of Ca^{2+}-dependent calmodulin-binding transcription activators (CAMTAs[3,4]) revealed that at least some of their DNA target sites coincide with such Ca^{2+}-responsive *cis* elements.[4] Detailed analysis of the CAMTA genes revealed their roles in biotic[5] and abiotic stresses,[6] growth control[6] and senescence [results to be presented]. Thus, CAMTAs may provide a link between Ca^{2+} signals and the transcription machinery in a variety of physiological responses, at all stages of the plant's life cycle, and in response to biotic and abiotic stresses.

1 Kaplan B. *et al* (2006) Plant Cell 18: 2733-2748
2 Finkler A. *et al* (2007) Plant Signaling and Behavior 2: 17-19
3. Bouché N. *et al* (2002) J Biol Chem 277: 21851-21861
4 Finkler A. *et al* (2007) FEBS Leters 581: 3893-3898
5 Galon Y. *et al* (2008) FEBS Letters 582: 943–948
6 Galon Y. *et al* (submitted).

P201 Analysis of expression and function of members of the transcription factor NF-Y family

Yanfang Wu, Andrea Voigt, Bernhard Grimm

Institute of Plant Physiology, Humboldt University, Berlin, Germany

In yeast and mammals, the transcription factor *NF-Y* is composed of the subunits A, B and C and binds to the CCAAT box cis-elements. While human and animals encode the three subunits of *NF-Y* each in single copy gene, *Arabidopsis thaliana* possess 10, 13 and 13 genes encoding the *NF-Y* subunits A, B and C, respectively. A few individual plant *NF-Y* subunits have been described to be involved in a number of important biological processes. Based on previous expression profiles of all *NF-Y* genes in *Arabidopsis thaliana* during development and in response to stress conditions, we selected some candidate genes for *NF-Y* subunits for detailed analysis. These *NF-Y* genes were analyzed on their cell-specific expression pattern during plant development by using promoter: GUS fusion gene constructs in stable transformed Arabidopsis plants. Histochemical analysis of GUS expression revealed the tissue-specific activities of these promoters during plant development. We used a reverse genetic approach to examine the functions of the *NF-Y* subunits under abiotic stress. Two T-DNA insertion mutants for the *NF-YB3* gene were analysed. One mutant carries the T-DNA in the *NF-YB3* 5´UTR and shows a low transcript level using semi-quantitative RT-PCR. The second mutant contains the T-DNA insertion in the *NF-YB3* coding sequence. A full length *NF-YB3* transcript was not detectable. The two mutants displayed delayed flowering under long-day conditions. Transcript analysis suggested that *NF-YB3* can promote flowering by enhancing expression of the *FT* gene. The mutants also showed insensitivity of ABA-inhibition of seed germination. ABA is a key component of various stress responses, such as cold, drought, osmotic or salt stress. ABA-induced transcriptional activation is currently investigated in the *nf-yB3* mutants and wild type under those abiotic stress conditions to elucidate the functions of NF-YB3 for plant responses to stress and during development.

P202 ROF2 is involved in thermotolerance via its interaction with ROF1

David Meiri, Odelia Farchi-Pisanty, Reut Cohen-Peer, Adi Avni, Adina Breiman

Tel Aviv University, Israel

The ROF2 (FKBP65) is a heat stress induced protein, which belongs to the FK506 Binding Protein (FKBP) family. It is homologous to ROF1 (FKBP62), which has recently been shown to be involved in long term acquired thermotolerance by its interaction with HSP90.1 and HsfA2.

In this study, we have demonstrated that ROF2 negatively regulates long term acquired thermotolerance. In the absence of ROF2, the small heat stress proteins (sHSPs) were highly expressed and the Arabidopsis seedlings were resistant to heat stress. It was further demonstrated that ROF2 is transcribed by HsfA2 which is also essential for the maintenance of ROF2 during recovery from heat stress. ROF2 localization to the nucleus was observed several hours after heat stress exposure. ROF2 was shown to interact with ROF1 and the heterodimers ROF1/ROF2 abrogate HsfA2 transcription activity. The immunosuppressive drug FK506 inhibited this interaction.

We suggest that ROF2 associates itself to the complex ROF1-HSP90.1- HsfA2 and propose a model for the mode of action of ROF2 as negative feedback regulator of HsfA2.

P203 Regulatory madness: The conflicting roles of microRNA-395 in sulfate assimilation

Colette Matthewman1, Cintia Kawashima2, Ruslan Yatusevich3, Tamara Gigolashvili3, Ulf-Ingo Flügge3, Tamas Dalmay2, Stanislav Kopriva1

1John Innes Centre, Norwich, UK, 2University of East Anglia, Norwich, UK, 3Der Universität zu Köln, Köln, Germany

Sulfate, an indispensable macronutrient for all organisms, is the major sulfur source required by plants for healthy growth. However, the availability of sulfate is often low in the environment, leading to reduced crop yield and quality. Sulfate assimilation produces a range of essential sulfur-containing compounds, such as amino acids, coenzymes and many secondary metabolites. ATP sulfurylase (ATPS) catalyses the initial sulfate activation step, required for sulfate assimilation, thus making it a prime candidate for further study. Genes encoding four ATPS isoforms have been identified in *Arabidopsis thaliana*, but roles and regulation of these separate isoforms remain to be determined. A new player in the regulation of *ATPS* was recently identified in Arabidopsis: microRNA-395 (miR395). The miR395 targets three *ATPS* genes, and the sulfate transporter SULTR2;1. The miR395 expression is up-regulated in response to sulfate deficiency, evidently being involved in the response to this nutrition stress. However, other components of sulfate assimilation are simultaneously up-regulated, so that the down-regulation of *ATPS* expression is directed against the regulation of the rest of the pathway. We are investigating the relationship between the miR395 and *ATPS* genes, to try and understand this confusing tale of regulatory madness.

P204 Detoxification of TNT by endogenous oxophytodienoate reductases, glutathione-S-transferases and glucosyltransferases in Arabidopsis

Liz Rylott, Emily Beynon, Fernando Gandia-Herrero, Helen Sparrow, Astrid Lorenz, Neil Bruce

CNAP, University of York, York, UK

Plants, as mostly sessile organisms, have evolved complex detoxification systems to deal with a diverse assortment of toxic chemicals present in the environment. The plasticity of this system also allows plants to detoxify relatively recently produced, synthetic pollutants such as explosives. 2,4,6-Trinitrotoluene (TNT) is a degradatively recalcitrant, toxic, contaminant that presents a serious and significant threat to the environment. Although global contamination levels are difficult to quantify, the US Department of Defense has estimated that the clean up of unexploded ordnance, discarded military munitions, and munition constituents on its active ranges, a total of 24.6 million acres, would cost between US$16 billion and US$165 billion.

Using microarray experiments we have identified, and now characterised, the involvement of three enzyme families in the classic activation and conjugation phases of xenobiotic detoxification. The Old Yellow Enzyme plant homologues oxophytodienoate reductases (OPRs) are a small family of enzymes, only one of which, OPR3, is of known physiological function, that of jasmonate biosynthesis. Studies have suggested that OYE homologues function as antioxidants, detoxifying the breakdown products of lipid peroxidation and other toxic electrophilic compounds. This oxidative stress could result from pathogen attack, wounding or exposure to xenobiotics. Here, we demonstrate a role for OPRs in the detoxification of the xenobiotic TNT presenting new data showing the OPR-catalysed activation of TNT. Following activation, we show that uridine diphosphate (UDP) glycosyltransferases (UGTs) conjugate TNT derivatives to sugars *in planta*, with marked differences in activity and stereospecificity. We also show the ability of glutathione reductases to conjugate the TNT molecule directly with release of nitrite.

P205 Function of CDPKs in abiotic stress signal transduction

Anja Liese, Britta Ehlert, Susanne Matschi, Sandra Franz, Joachim Kurth, Tina Romeis

FU Berlin, Germany

Calcium-dependent protein kinases (CDPK) are unique Ser/Thr protein kinases that are implicated in the perception of stress-specific changes in the cytoplasmatic calcium concentrations and their translation into phosphorylation signals in plants. We identified by comparative physiological assays and expression studies between *Arabidopsis thaliana* Col-0 wild type and *cpk* T-DNA insertion lines several isoforms, whose activity is required for abiotic stimulus-induced stress responses. Among these, *cpk21* plants exhibit an altered behaviour in drought and hyper-osmotic stress tolerance and growth. Corroborated by expression data and metabolite analysis our data suggest that CPK21 functions as negative regulator early during the onset of the plant abiotic stress response. For biochemical characterisation different variants of CPK21, including single and double EF-hand motif-mutations, were transformed in the *cpk21* mutant background. The C- and N-terminal EF-hand pairs can be distinguished in their biochemical calcium-dependent enzyme activities and differ in their functionality in plant stress tolerance. Furthermore, calcium-dependent *in vitro* and *in vivo* enzyme activity not only correlated with

the biological function of CPK21 but also enables approaches to identify *in vivo* phosphorylation substrates for the enzyme.

P206 Post-translational regulation in the Arabidopsis circadian clock

Rachel Green

Hebrew University, Jerusalem, Israel

As an adaptation to life in a world with predictable daily changes, plants have endogenous circadian (~24 hour) clocks that regulate a diverse range of cellular and physiological events from gene expression and protein phosphorylation to cellular calcium oscillations, hypocotyl growth, leaf movements and photoperiod-dependant flowering. In Arabidopsis, as in other model organisms, circadian rhythms are generated by molecular oscillators that consist of interlocking feedback loops involving a number of elements. CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) and LATE ELONGATED HYPOCOTYLS (LHY) are closely-related single MYB transcription factors that have been identified as key elements in the Arabidopsis oscillator. Research in other model organisms has shown that post-translational regulation of oscillator components plays a critical role in the generation of the ~24 hour cycles. Using epitope and fluorescence tagged CCA1 and LHY proteins in transgenic plants, we are examining the interactions and sub-cellular localization of CCA1 and LHY. We discuss our findings in the context of the functioning of the Arabidopsis oscillator.

P207 Dynamic interactions of vernalization and photoperiod determine local adaptation of flowering time independent of latitude in *Arabidopsis thaliana*

A M Lewandowska-Sabat1, S Fjellheim1, P Winge2, A Bones2, T Sparstad2, C G Fossdal3, J E Olsen1, O A Rognli1

1Norwegian University of Life Sciences, IPM, Aas, Norway, 2Norwegian University of Science and Technology, Trondheim, Norway, 3Norwegian Forest and Landscape Institute, Aas, Norway

Regulation of flowering time in *Arabidopsis thaliana* is controlled by a network of pathways integrating environmental and internal signals. Two of these pathways, the vernalization and photoperiodic pathways, mediate responses to prolonged cold period and photoperiod, respectively.

A number of *A. thaliana* populations from high-latitude and high-altitude locations in Norway were collected and phenotyped for flowering time in response to 5 photoperiods and 5 vernalization treatments.

Vernalization and photoperiodic sensitivity were not correlated with latitude but rather with climatic factors such as winter temperature and precipitation that do not vary with latitude, especially in coastal environments. Coastal populations, both from subarctic and intermediate latitudes, were rather insensitive towards the length of the vernalization treatment but very sensitive towards differences in photoperiods. Stronger photoperiod sensitivity in coastal populations might be a necessary adaptation for sensing the onset of spring in regions with relatively mild and unpredictable winter climates as opposed to continental climates with more stable winters.

FLC sequence variation was only partly associated with vernalization response, whereas variation in transcript levels of *CRY2, TOC1* and *GI* was correlated with photoperiodic responses. This suggests that local adaptation of populations may be partly mediated by photoreceptors and circadian clock pathways.

P208 The dynamic nature of molecular responses to cold and their interaction with diurnal regulation

Carmen Espinoza1, Takayuki Tohge1, Andrea Leisse1, Thomas Degenkolbe1, Alisdair R Fernie1, Lothar Willmitzer1, Dirk K Hincha1, Matthew A Hannah2

1Max-Planck-Institut für Molekulare Pflanzenphysiologie, Golm, Germany, 2Current address: Bayer BioScience, Gent, Belgium

Low temperature is a major challenge to plant growth and survival. Many plants are able to adapt to this by increasing their freezing tolerance in a process known as cold acclimation. Our aim is to dissect the molecular basis of cold acclimation and to characterize the underlying regulatory networks. Our previous work, via the analysis of a large number of published studies, revealed that diurnal and circadian-regulated genes are responsible for the majority of variation between experiments to identify cold-responsive genes, even though paired diurnal controls were used. Using targeted expression analyses we showed that this is mainly because cold dampens or disrupts the cycles of many clock components and output genes. These data indicated that understanding the response of plants to low-temperature required the consideration of diurnal effects. We therefore challenged the accepted model of cold acclimation - a transition from the non-acclimated to the cold-acclimated molecular state - by molecular profiling of diurnal time series at normal and low temperature. Consistent with our model for transcripts, observed metabolite changes are also time-of-day dependent. Our data also establish a new model for how metabolism responds during cold acclimation. Conventional stress metabolites accumulate and this is consistent with pathway regulation at the transcriptional level, however, we also observe a more extensive metabolic reprogramming which is dynamic, interacts with circadian and diurnal regulation and is largely controlled at the post-transcriptional level.

P209 Transcription factors coordinating the salt stress response mediated by the OsRMC peptide: Identification and characterization

Tania Serra, Ana Paula Santos, Margarida Oliveira, Nelson Saibo

ITQB/IBET, Oeiras, Portugal

Adverse environmental conditions, such as drought and high salinity, restrain plant ability to survive and reproduce worldwide. High salinity deleterious effects are due to

ionic and osmotic imbalance in the cell, which eventually cause growth and yield reduction. Adaptation to abiotic stress implies the perception and transduction of stress signals through specific pathways.

Transcription factors (TFs) play a significant role in stress response networks since they coordinate the expression of stress-responsive genes. *OsRMC* encodes an apoplast peptide whose expression is induced by jasmonic acid (JA) and salt stress. This peptide inhibits both the JA and salt response signaling pathways, probably through the interaction with one or more stress-responsive receptors.

The aim of this work is to identify and characterize TFs modulating the expression of *OsRMC* under salt stress conditions in rice. The yeast one-hybrid system was used to screen a salt-induced rice cDNA expression library for novel TFs. The interaction between the OsRMC promoter region and the DNA-binding proteins identified will be validated through gel shift assays. TFs characterization includes the analysis of their expression under several abiotic stress conditions (e.g. cold, salt, drought, ABA), *in vivo* localization and transcriptional activity.

Arabidopsis mutants for orthologs of the novel TFs and also transgenic Arabidopsis will be used to assess the TFs biological function.

P210 Functional dynamics in plant mitochondrial metabolism

ChunPong Lee, Holger Eubel, Nicolas L Taylor, A Harvey Millar

The University of Western Australia, WA, Australia

Heterogeneity of mitochondria between cell, tissue and organ types is critical for respiratory metabolism and peripheral mitochondrial pathways to integrate into the function of their host cells. This phenomenon may also be important to understand tissue-specific phenotypes of mitochondrial dysfunction: e.g. in animals many mitochondrial diseases yield tissue or organ specific symptoms and in plants CMS lines and knockouts of nuclear-encoded mitochondrial components also yield developmental/tissue selective abnormalities. This phenomenon could also be a key to uncovering the mechanisms that regulate mitochondrial biogenesis, the steady state maintenance of mitochondrial function over prolonged periods of time, and even the cyclic alteration of mitochondrial functions in response to light, temperature and abiotic stress. We will present details of new proteomics experiments uncovering the quantitative comparison of shoot, root, silique, flower, stem and cell culture mitochondria, a ten time point study of the diurnal changes in shoot mitochondria and new data on the oxidative stress induced changes in the mitochondrial proteome. These have been linked to information on transcription differences and the study of the functional consequences on these changes to enzyme activities in attempts to more broadly understand the drivers and the impact of changes in protein abundance on mitochondrial metabolic function.

P211 Implication of CLCc in salt stress resistance in *Arabidopsis thaliana*

Mathieu Jossier1,2, Benoît Guillemardet1, Hélène Barbier-Brygoo1, Geneviève Ephritikhine1,2, Sophie Filleur1,2

1Institut des Sciences du Végétal, CNRS UPR 2355, Laboratoire Transporteurs Transmembranaires et Signalisation, 91198 Gif-sur-Yvette, France, 2Université Paris-Diderot, 75205 Paris, France

Salinity is a one of the major constraints of crop productivity because it reduces yield and limits expansion of agriculture. Plant salt resistance requires cellular ion homeostasis involving Na^+ and Cl^- uptake and compartmentalization in the vacuole. Untill now, most of the salt resistance transporters identified in this uptake are Na^+ transporters. We report here for the first time the implication of an anion transporter/channel in salt long term resistance.

The *A. thaliana* CLCc belongs to the ChLoride Channel (CLC) family of anion transport proteins. Previous results have implicated CLCc in nitrate accumulation like the well characterised CLCa, a nitrate/H^+ antiporter located on the tonoplast (Harada *et al*, 2004, *J. Ex. Bot.* 55, 2005-14; De Angeli *et al*, 2006, *Nature* 442, 939-42). However, recent results suggest that CLCc could be selective for chloride instead of nitrate as the highly conserved S168 in CLCa responsible for NO3 selectivity is replaced by a proline in CLCc (Zifarelli *et al*, 2009, *EMBO* 28, 175-82; Bergsdorf *et al*, 2009, *JBC* 17, 11184-93).

Using GFP fusion, we localized CLCc on the tonoplast like CLCa in *Arabidopsis thaliana* cell culture protoplasts. First results suggest a role of CLCc in salt stress resistance as *A. thaliana* knock out mutants of *CLCc* present a hypersensitivity to ionic stresses. The phenotypic characterization of these mutants will be presented in details.

P212 Adaptation of Arabidopsis leaves to moderate drought stress depends on their developmental stage

Aleksandra Skirycz1, Stefanie de Bodt1, Toshihiro Obata2, Alisdair Fernie2, Dirk Inze1

1Department Plant Systems Biology, VIB, and Ghent University, Gent, Belgium, 2MPI for Molecular Plant Physiology, Potsdam-Golm, Germany

It was demonstrated that water limitation leads to a rapid reduction in leaf growth and this is thought to be an important adaptation response to save and re-distribute resources to avoid them becoming limited. This initial decline in growth rate is followed by growth recovery and adaptation to the new conditions.

The main aim of this work was to investigate, using a combination of growth and molecular profiling analysis, how growing *Arabidopsis thaliana* leaves adapt to prolonged mild water deficit and compare the molecular responses to those occurring in mature tissues. We first established a stress set-up that reduces final leaf size by 50% without altering the developmental timing of the

formation of new leaves. Kinematic analysis of leaf growth clearly demonstrated that leaves that initiate and develop under mild drought have fewer and smaller cells yet both cell division and expansion can very well adapt to stress conditions. Subsequently, we microdissected leaves at an early stage when only cell proliferation occurs; at a stage when leaf cells are mainly expanding; and a mature stage in which cells are fully differentiated and subjected them to transcriptome and metabolome analysis. The obtained data clearly demonstrate that proliferating, expanding and mature tissues each respond in a unique manner to mild drought stress; several hundreds transcripts being specific for growing leaves. Interestingly, mature leaves show the classical stress response as determined in previous whole plant studies, yet they are particularly distinct when compared to growing tissues. Mechanisms underlying growth adaptation of Arabidopsis leaves to water stress such as re-programming of mitochondrial metabolism occurring specifically in the proliferating leaves will be discussed.

P213 A cytosolic ABA receptor

Arthur Korte, Yue Ma, Izabela Szostkiewicz, Alexander Christmann, Erwin Grill

Lehrstuhl für Botanik, Technische Universität München, Am Hochanger 4, D-85354 Freising, Germany

The plant hormone abscisic acid (ABA) acts both as a developmental signal and as an integrator of environmental cues such as drought and cold that affect the plant's water status. ABA responses redirect gene expression, reduce transpiration, protect photosynthesis, and control plant growth. Key players in ABA signal transduction include the PP2C protein phosphatases ABI1 and ABI2 that act by negatively regulating the entire range of ABA responses. We have identified an interactor of ABI1 and ABI2 that we have named regulatory component of ABA receptor 1 (RCAR1). We then tested RCAR1 for its capacity to regulate ABI1 and ABI2. In the presence of RCAR1, ABA inactivated the phosphatase activity of both enzymes with apparent dissociation constants for S-ABA in the nanomolar range. The enantiomer *R*-ABA and the stereoisomer *trans*-ABA were more than two orders of magnitude less effective in inhibiting the PP2Cs in accordance with their lower physiological activity. Using ABA binding assays, we could show specific interaction of S-ABA to RCAR1. Enhanced expression of *RCAR1* in Arabidopsis protoplasts or transgenic plants affected all facets of ABA signaling including gene expression, stomatal regulation, seed germination, and vegetative growth. In addition, the downregulation of *RCAR1* in protoplast using RNAi-constructs significantly reduce the ABA signaling. We conclude that the complex between the PP2Cs and RCAR1 has the hallmark of an ABA receptor in that it can selectively recognize and transduce the ABA signal. Binding of ABA to the receptor complexes inactivates the phosphatases that globally repress ABA responses, thereby activating the entire range of physiological processes regulated by ABA.

P214 The Arabidopsis C/S1 bZIP transcription factor network controls reprogramming of metabolic gene expression in response to energy deprivation

Katrin Dietrich1, Fridtjof Weltmeier1, Andrea Ehlert1, Johanna Lendner1, Klaus Harter2, Wolfgang Dröge-Laser1

1Albrecht-von-Haller-Institut, Universität Göttingen, Untere Karspüle2, D-37073 Göttingen, Germany, 2Zentrum für Molekularbiologie der Pflanzen, Pflanzenphysiologie, Auf der Morgenstelle1, 72076 Tübingen, Germany

In plants, energy deprivation leads to dramatic reprogramming of transcription. In particular, amino acid (aa) metabolism is strongly affected. Growth under extended night conditions leads to transcriptional and post-transcriptional activation of the group S1 basic leucine zipper transcription factor AtbZIP1. Gain- and loss-of-function approaches in transgenic plants and studies in protoplasts define AtbZIP1 and its nearest homologue AtbZIP53 as crucial regulators of gene expression during starvation response.[1] Depending on these bZIP transcription factors, expression of aa metabolic genes is altered which can be correlated with the levels of the corresponding aa, in particular Pro, Asn, Gln, Val, Leu and Ile. Gene regulation is mediated in a G-box dependent manner. Direct binding of the bZIP transcription factors to promoters of aa metabolic genes has been confirmed by Chromatin Immunoprecipitation (ChIP). Since knock-out approaches lead only to a partial reduction of gene activation, bZIP heterodimers are postulated to be functionally interlinked in energy balance control. A network of specific bZIP heterodimers consisting of group C and S1 bZIPs has been identified[2,3] and its function in reprogramming gene expression in starvation response has been studied.

1 Weltmeier *et al* (2009) PMB 69: 107-119.
2 Weltmeier *et al* (2006) EMBO J. 25:3133 – 3143.
3 Ehlert *et al* (2006) Plant J 46:890 – 900.

P215 The endogenous *GL3*, but not *EGL3* gene, is necessary for anthocyanin synthesis as induced by nitrogen depletion in Arabidopsis rosette stage leaves

Dugassa Feyissa1, Trond Løvdal1, Kristine Olsen1, Rune Sliimestad2, Cathrine Lillo1

1University of Stavanger, Stavanger, Norway, 2PlantChem, Sørheim, Klepp, Norway

The bHLH transcription factors EGL3 (ENHANCER OF GLABRA3) and its close homologue GL3 (GLABRA3) are important regulators of the anthocyanin pathway in *Arabidopsis thaliana*, and together with TTG1 (a WD40 repeat protein) and MYB transcription factors regulate specific genes in the pathway. In response to nitrogen depletion, the MYB genes *PAP1/PAP2 (PRODUCTION OF ANTHOCYANIN PIGMENT1/2)* and *GL3* are strongly induced, and anthocyanin synthesis is activated in seedlings and rosette stage plants. In this study we show that anthocyanins accumulate in both wild type and *egl3*, but not in *gl3* loss-of-function mutants when depleted of

nitrogen. Several structural genes of flavonoid metabolism including *CHS (CHALCONE SYNTHASE), FLS1 (FLAVONOL SYNTHASE1)* and *ANS (ANTHOCYANIDIN SYNTHASE)*, were induced in response to nitrogen depletion in wild type as well as in the *egl3* and *gl3* mutants. Strikingly, in the *gl3* mutant *DFR (DIHYDROFLAVONOL 4-REDUCTASE)* transcript level was only 2% of the levels in wild type or *egl3* mutant. Hence, low expression of *DFR* appears to be the bottleneck preventing anthocyanin synthesis in the *gl3* mutant. The specific effect on *DFR*, but not *ANS*, is compatible with involvement of the MYB2L inhibitor.

1 Lea US, Slimestad R, Smedvig P, Lillo C (2007) Nitrogen deficiency enhances expression of specific MYB and bHLH transcription factors and accumulation of end products in the flavonoid pathway. Planta 22: 1245-1253

2 Lillo C, Lea US, Ruoff P (2008) Nutrient depletion as a key factor for manipulating gene expression and product formation in different branches of the flavonoid pathway. Plant, Cell Environ 31: 587-601

P216 AtGTL1 transcription factor regulates drought tolerance and water use efficiency

Chan Yul Yoo1, Jing Bo Jin2, Kenji Miura3, Mike Gosney1, Yinhua Jin2, Paul M Hasegawa1, Michael V Mickelbart1

1Purdue University, West Lafayette, IN, USA, 2Institute of Botany, Chinese Academy of Sciences, Beijing, China, 3University of Tsukuba, Tsukuba, Japan

Improving drought tolerance and water-use efficiency (WUE) are global priorities to develop crops with higher biomass production or yield using less water because of decreasing fresh water availability and increasing agricultural drought. However, the genetic basis for how plants regulate WUE is not known. Genome-wide screening of expression libraries using recombinant CaM has revealed that AtGT-2 (GT element-binding transcription factor) family members contain putative CaM-binding domains implicating their function in Ca^{2+}/CaM signaling. In this study, we have determined that AtGTL1, one member of the AtGT-2 family, regulates WUE and drought tolerance through a mechanism(s) that affects stomatal density and transpiration. *gtl1* T-DNA insertional mutations (*gtl1-1*, *gtl1-2*, and *gtl1-3*) substantially improve WUE of plants without inhibiting biomass accumulation. Further, the mutations enhance the capacity of plants to survive water deficit stress by facilitating the maintenance of leaf relative water content by reducing transpiration. Expression of *AtGTL1* decreased with dehydration, which is consistent with the notion that GTL1 is a negative regulator of WUE and drought tolerance. Leaf stomatal density is lower in *gtl1* plants. Together, the results indicate that *gtl1* mutations reduce transpiration by regulating stomata number in leaves. Whole-genome expression analysis using an Affymetrix gene chip and RT-PCR analysis revealed that expression of *STOMATAL DENSITY AND DISTRIBUTION 1 (SDD1)* is up-regulated in *gtl1*, which may be the cause of reduced leaf stomatal density. We hypothesize that GTL1 regulates WUE and drought tolerance through mechanisms that are linked to *SDD1* expression and reduced stomatal index.

P217 Dissonant circadian clock in early-standing magnesium deficient leaves

Christian Hermans1, Marnik Vuylsteke2, Dirk Inzé2, Nathalie Verbruggen1

1Laboratory of Plant Physiology and Molecular Genetics, Université Libre de Bruxelles, 1050 Brussels, Belgium, 2Department of Plant Systems Biology, VIB & Department of Plant Biotechnology and Genetics, Ghent University, 9052 Gent, Belgium

The earliest symptoms observed within days to weeks of Mg deficiency consist of the impairment of sugar partitioning and the enhancement of antioxidative mechanisms, before any chlorophyll breakdown. Knowledge on earlier impact or other processes is scarce. Therefore, we carried out a genome-wide transcriptomics analysis in *Arabidopsis thaliana*. The response to short-term (04, 08 and 28h after the removal of Mg from the nutrient solution) and long-term deprivation (7 days + 08h and 7 days + 24h) were studied in 5-weeks-old plants grown hydroponically. Mg starvation triggered an asynchronous and different response with an increased number of differentially expressed genes after 8 h in roots and after 28 h in young mature leaves. Interestingly, the earliest major change in behaviour in the roots was credited to the master clock controller *CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1)* gene. After 7 days, the circadian clock in leaves was deregulated in its turn. Early morning genes, such as *CCA1* and *LHY1* extended their expression over the light period, and evening genes, such as *APRR1/TOC1*, *APRR3, APRR5, ELF4* and *GI*, disrupted in their rhythmic expression when compared to control plants in which Mg was fully supplied. Here, we discuss the consequences for Mg-deficient plants with a dissonant circadian clock, in particular in term of the ruining of the photosynthetic apparatus.

P218 Analyzing the molecular differences of the *SPA* genes that cause distinct functions - The dark side of SPA2

Kirsten Fittinghoff, Ute Hoecker

University of Cologne, Germany

In Arabidopsis, the COP1-SPA ubiquitin ligase complex plays a central role in suppressing light signaling. The COP1-SPA complex targets positively acting factors such as HY5, several photoreceptors and the flowering time regulator CONSTANS for degradation via the 26S proteasome. While *COP1* is a single copy gene, the SPA proteins are encoded by four different loci (*SPA1-SPA4*). All SPA proteins have redundant, but also distinct functions in regulating plant development. We could show that distinct functions of the *SPA* genes partially correlate with their distinct gene expression patterns. However, differences in *SPA* gene expression cannot account for all distinct *SPA* gene functions. Promoter-swap experiments with *SPA1, SPA2* and *SPA4* show that all SPA proteins are potent repressors in dark-grown seedlings. SPA1 and SPA4 also act as repressor in the light. *SPA2*, however, can never act in light-grown seedlings, even not when it is expressed from the strong light-induced *SPA1* promoter. These results show that SPA proteins themselves

feature properties that contribute to the characteristic SPA protein functions.

P219 Analysis of suppressor mutants of a PARN deficient mutant, *ABA hypersensitive germination2-1*

Takashi Hirayama1,2, Shimpei Hayashi2

1RIKEN, Yokohama, Japan, 2Yokohama City Univ., Yokohama, Japan

ABA hypersensitive germination2-1 shows pleiotropic phenotypes including an ABA hypersensitivity, an SA hypersensitivity, and a dwarf phenotype.[1] The *ahg2-1* mutation seems to reduce the expression of the polyA specific ribonuclease. To address the molecular basis of the AHG2/PARN function and the effect of the *ahg2-1* mutation, we conducted a genetic screen for suppressor mutants of *ahg2-1* using root elongation as a physiological marker. We isolated a dozen candidates with various suppressor strengths. Interestingly, most of the suppressor mutants suppressed all the *ahg2-1* phenotypes, ABA and SA hypersensitivities, and dwarf phenotype, suggesting that the functions of these suppressors and AHG2/PARN are closely related. We identified the corresponding gene for one of the suppressor mutants. Predicted function of the gene products seem closely related to that of AHG2/PARN. We will discuss the mechanisms by which *ahg2-1* causes such the diverse and complicated effects.

Nishimura *et al*, Plant J., 2005.

P220 Involvement of amine oxidases in nitric oxide (NO) biosynthesis and in NO-mediated abiotic stresses

Rinukshi Wimalasekera1, Corina Villar2, Tahmina Begum3, Günther Scherer1

1Institute of Plant Molecular Physiology, Leibniz University of Hannover, Herrenhäuser Str.2, D-30419 Hannover, Germany, 2Institute of Plant Research, ETH, Zürich, Switzerland, 3ZMBP, University of Tübingen, Germany

Nitric oxide (NO) biosynthesis is regulated by a variety of biotic and abiotic signals. When Arabidopsis seedlings were treated with polyamines (PA) putrescine, spermidine and spermine, increased extracellular accumulation of NO was observed by fluorimetry using cell-impermeable NO binding dye diaminorhodamine-4M. In comparison to wild type (WT), T-DNA insertional knockouts defect in polyamine catabolizing copper-containing amine oxidases (*CuAO*) and FAD containing polyamine oxidases (*PAO*) showed relatively lower NO accumulation in response to PA treatment. Fluorescence microscopic observations using cell-permeable NO-binding dye diaminorhodamine-4M acetoxymethyl ester, showed enhanced NO biosynthesis in elongation zone of the root tips and primary leaves especially in the veins and trichomes of PA treated WT, *Cuao* and *pao* knockout seedlings. PA induced NO production in the root tips of *Cuao1* knockouts was significantly lower than in the WT. NO mediated posttranslational protein modification through S-nitrosylation was detected by biotin switch method. PA treatment enhanced the intensity of S-nitrosylated proteins. In addition to polyamines, an increase in NO release was observed in the ABA and NaCl treated WT and knockout seedlings. However, the relative NO production was lower in Cuao1 and *pao2* in response to ABA and NaCl respectively. Compared to WT, *Cuao1* showed less inhibition of primary root elongation in response to exogenous ABA and mannitol application. Taken together, the results suggest that involvement of PA and amine oxidases in regulation of NO biosynthesis and in root development during stress conditions.

P221 DNA damage signaling controls the expression level of B2-type CDK in *Arabidopsis thaliana*

Sumiko Adachi, Masaaki Umeda

Graduate School of Biological Sciences, Nara Institute of Science and Technology, Ikoma, Japan

Genome integrity is maintained by coupling DNA damage repair to cell cycle progression. Plants lack orthologs of some of the genes, which in animal cells are important for the DNA damage checkpoint, indicating that they may have distinct mechanisms to cope with genotoxic stress. The eukaryotic cell cycle is controlled by cyclin-dependent kinases (CDKs). Arabidopsis CDKs have been classified into six types, among which the A- and B-type CDKs are thought to be crucial for cell cycle progression. The B-type CDKs (CDKBs) are plant-specific and are further classified into two subtypes, CDKB1 and CDKB2. CDKBs are expressed specifically from late S- to M-phase. We have recently reported that the abundance of Arabidopsis CDKB2 protein is regulated not only at the transcriptional level, but also through proteasome-mediated protein degradation. We now show that the accumulation of CDKB2 is further suppressed in response to DNA double-strand breaks, suggesting a regulatory mechanism that inhibits G2-to-M-phase progression when DNA damage has occurred.

P222 Phosphatidic acid regulates protein kinases in Arabidopsis responses to salt

Fionn McLoughlin, Carlos Galvan-Ampudia, Christa Testerink

University of Amsterdam

Phosphatidic acid (PA) has been identified as a lipid second messenger in both plants and animals. In plants, PA accumulates in response to several biotic and abiotic stress stimuli, such as drought, salinity, cold, wounding and pathogen infection. Although PA's mode of action is not clear yet, an important discovery is that several protein kinases appear to have PA-binding affinity. These include the phosphoinositide-dependent protein kinase 1 (PDK1), PINOID protein kinase (PID), the negative regulator of ethylene signalling CTR1, and two individual SNF1-related protein kinases (SnRK2.4 and 2.10).

The SnRK2 subfamily is plant specific and most family members were shown to be activated by salt in protoplasts. Using hydroponically grown *A. thaliana* plants, we were able to instantly monitor SnRK2 activity and study their localization in root tissue in response to salt

treatment. Interestingly, not all SnRK2 isoforms have affinity for PA. Knock-out mutants of both PA-binding isoforms, as well as a double mutant, are currently being characterized with respect to salt tolerance.

Another line of research focuses on the role of phosholipid signaling in directing osmotic stress-induced loss of gravitropism. We observed that osmotic stress in roots induces changes in the subcellular localization of signalling proteins, including the protein kinases PINOID and PDK1. We hypothesize that a signalling cascade involving phospholipid signalling and protein phosphorylation directs root growth away from high salt concentrations. Currently, mutants in *pid, pdk1* and the phospholipid-metabolizing enzymes that generate PA, are being tested in a salt avoidance assay.

P223 Is ZIF1, a critical component of Zn and Fe homeostasis, a nicotianamine transporter?

Mike Haydon, Ute Kraemer
University of Heidelberg, Germany

Metal ions, such as Zn and Fe, are essential for cellular metabolism and development, but are toxic when present in excess. To effectively deliver these to the desired targets, while tightly controlling the concentrations of free metal ions, plants use a combination of organic chelators and compartmentalisation to sequester excess metals. This requires various membrane transport proteins for free metal ions, low-molecular-weight chelators and metal-chelator complexes, yet the identity and substrates of many of these proteins remains unclear. *ZIF1* was identified based on a Zn-hypersensitive mutant phenotype in *A. thaliana.* The gene encodes a tonoplast-localised major facilitator superfamily (MFS) membrane protein, which transport organic substrates. Therefore, it has been proposed that ZIF1 transports a metal chelator into the vacuole. *ZIF1* is up-regulated in plants grown in media containing excess Zn or lacking Fe, and *zif1* mutants are hypersensitive to Fe deficiency. A detailed characterisation of *zif1* mutants and *ZIF1* over-expressors indicates a potential dual role for *ZIF1* in coping with Zn excess and Fe limitation. Over-expression of *ZIF1* confers a remarkable inter-veinal chlorosis in leaves that phenocopies mutant or transgenic plants that are deficient in nicotianamine (NA), a key metal chelator in plants. In addition to Zn accumulation in root vacuoles, *35S-ZIF1* plants exhibit increased root:shoot ratios of Zn concentrations, systemically up-regulated Zn- and Fe-deficiency responses and up to 10-fold elevated root NA concentrations. Together, these results are consistent with a role for *ZIF1* in vacuolar metal sequestration by transporting NA across the tonoplast. Current experiments aim to directly demonstrate a contribution of *ZIF1* to partitioning of NA and metals into the vacuole. Furthermore, to identify genes involved in the metal-dependent regulation of *ZIF1*, a forward genetic approach is being used. Mutants have been identified that appear to be strongly impaired in Zn tolerance and/or Fe acquisition, suggesting that this approach could identify central regulatory components of metal homeostasis in plants.

P224 Role of the Ca^{2+}-dependent protein kinase CPK3 in the Arabidopsis salt-stress response

Norbert Mehlmer, Daniela Hofmann-Rodrigues, Bernhard Wurzinger, Andrea Mair, Markus Teige
Max F. Perutz Laboratories, University of Vienna, Austria

Plants have developed multiple strategies to adapt to changes in their environment. Extracellular signals could elicit changes in the cellular Ca^{2+} concentrations in plants[1,2] or activate protein kinase signalling.[3,4] Decoding of Ca^{2+} signals is performed by protein kinases by either direct protein phosphorylation or indirectly by changing gene expression patterns. Here we describe mechanisms to adaptation to salt stress by different signalling pathways in Arabidopsis and show that a CDPK has an essential function for salt stress adaptation. CDPK activity was strongly activated by salt stress and knockout mutants showed a salt sensitive phenotype comparable to MAPK knockout mutants. However, these signaling pathways seem to act independent. Salt-induced transcriptional induction of known stress-regulated marker genes was not affected by the CDPK pathway, whereas post-translational protein phosphorylation patterns revealed clear differences. Together with the different subcellular localization of MAPKs and CDPKs,[5] this indicates a different and independent mode of action in the salt stress response.

1 Harper, J.F., Breton, G., and Harmon, A. (2004). Ann. Rev Plant Biol.55, 263-288.
2 Cheng, S.H., Willmann, M.R, Chen, H.C., and Sheen, J. (2002). Plant Physiol. 129, 469-485.
3 Chinnusamy, V., Schumaker, K., and Zhu J.K. (2004). J. Exp. Bot. 55, 225-236.
4 Teige, M., Scheikl, E., Eulgem, T. *et al* (2004). Mol. Cell 15, 141-152.
5 Benetka, W., Mehlmer, N., Maurer-Stroh S., Sammer, M., Koranda, M., Neumüller, R., Betschinger, J., Knoblich, J.A., Teige, M., and Eisenhaber, F. (2008). Cell Cycle 7(23). [Epub ahead of print]

P225 The major flowering time gene, *Flowering Locus C*, regulates seed germination in *Arabidopsis thaliana*

George C K Chiang1, Deepak Barua1, Elena Kramer1, Richard Amasino2, Kathleen Donohue3
1Harvard University, Cambridge, MA, USA., 2University of Wisconsin-Madison, Madison, WI, USA, 3Duke University, Durham, NC, USA

FLOWERING LOCUS C (FLC) is a major regulator of flowering responses to seasonal environmental factors. Here we document that *FLC* also regulates another major life-history transition—seed germination—and that natural variation at the *FLC* locus and in *FLC* expression is associated with natural variation in temperature-dependent germination. *FLC*-mediated germination acts through additional genes in the flowering pathway—*FT, SOC1,* and *AP1*—before involving the ABA catabolic pathway (via *CYP707A2*) and GA biosynthetic pathway (via *GA20ox1*) in seeds. Furthermore, *FLC* regulation of germination is

largely maternally controlled, with *FLC* peaking and *FT*, *SOC1*, and *AP1* levels declining at late stages of seed maturation. High *FLC* expression during seed maturation is associated with altered expression of hormonal genes (*CYP707A2* and *GA20ox1*) in germinating seeds, indicating that gene expression before the physiological independence of seeds can influence gene expression well after any physical connection between maternal plants and seeds exists. The major role of *FLC* in temperature-dependent germination documented here reveals a much broader adaptive significance of natural variation in *FLC*. Pleiotropy between these major life stages therefore likely influences patterns of natural selection on this important gene, making *FLC* a promising case for examining how pleiotropy influences adaptive evolution.

P226 Ethylene plays a role in the root phenotype of the *arm* mutant

Christian Hermans1, Simona M Cristescu2, Frans J M Harren2, Filip Vandenbussche3, Dominique Van Der Straeten3, Nathalie Verbruggen1, Daniel R Bush4

1Plant Physiology and Molecular Genetics, Université Libre de Bruxelles, Belgium, 2Life Science Trace Gas Facility, Radboud University Nijmegen, the Netherlands, 3Unit Hormone Signaling and Bio-Imaging, Ghent University, Belgium, 4Biology Dpt., Colorado State University, Fort Collins, CO, USA

A screen for abnormal root architecture responses to high nitrate (repressing lateral root elongation) in the growth medium was carried out for a population of EMS-mutagenized Arabidopsis. We isolated the arm (anion altered root morphology) mutant showing conspicuous features conditional on high nitrate: short primary root, high number of laterals, radial swelling and high root hair density. A point mutation was identified in *AtCTL1* encoding a chitinase-like protein, for which mutant phenotypes were reported to implicate ethylene overproduction. Therefore, we asked whether ethylene production depends on nitrate abundance, and if the *arm* phenotype is the result of increased production of this hormone or altered sensitivity toward it.

To answer this question we have employed a laser-based ethylene detector in combination with an automated gas handling system. The instrument (type ETD-300; Sensor Sense BV, Nijmegen, NL) is a state-of-the art detector based on laser photoacoustic spectroscopy that is able to detect on-line about 300 pptv of ethylene within a 5s time scale, which is two orders of magnitude better than gas chromatography. We confirmed the ethylene overproducing mutant phenotype previously reported and found that production rate of *arm* was higher than of the wt at low nitrate, while no significant difference was observed at high nitrate. Ag^+ addition suppressed the radial swelling and high root hair density and partially increased the primary root length.

In contrast, no visible enhancement of symptoms was apparent through the action of ACC, suggesting that the ethylene signalling in *arm* is saturated at high nitrate.

P227 Osmotic stress-induced signals control root growth

Carlos S Galvan Ampudia1, Christine Zalejski2, Laszlo Bogre2, Remko Offringa3, Christa Testerink1

1University of Amsterdam, Swammerdam Institute for Life Sciences, Science Park 904, 1098 XH Amsterdam, The Netherlands, 2School of Biological Sciences, Royal Holloway, University of London, Egham, Surrey TW20 0EX, UK, 3Molecular & Developmental Genetics, Institute of Biology, Leiden University, Clusius Laboratory, Wassen-aarseweg 64, 2333 AL Leiden, The Netherlands

Plant roots are constantly exposed to a variety of abiotic stresses. Depending on the type and intensity of the stress, plants have the capability to overcome the stress by initiating signal transduction pathways that lead to the activation of ion transporters and expression of genes involved in tolerance. An alternative strategy is to modify root growth in order to avoid the stress source. Although it is known that environmental signals cause local changes in auxin distribution necessary for the reorientation of growth, the molecular basis of the avoidance strategy is still poorly understood.

Using physiological assays to measure salt avoidance, and live confocal microscopy of salt-stressed Arabidopsis roots, we set out to find the key players involved. Upon osmotic stress, phospholipid signals are produced, in particular phosphatidic acid (PA) and PIP2. The same lipids have been shown to activate 3-phosphoinositide-dependent kinase (PDK1), a master regulator of AGC protein kinases (Anthony *et al*, 2004) and its target PINOID (PID) (Zegzouti *et al*, 2006), a key regulator of polar auxin transport. Here, we show that osmotic stress in roots leads to rearrangements of the microtubule (MT) network and induces changes in the localization of signalling proteins, including PID and PDK1. Currently, mutants in *pid*, *pdk1* and the phospholipid-metabolizing enzymes that generate PA, are being tested in a salt avoidance assay. We propose a model on how osmotic stress regulates root growth through a signalling cascade in which phospholipid signalling and protein phosphorylation play a central role.

P228 A role for carbon metabolism in leaf growth response to soil water deficit? An integrated perspective

Irène Hummel1, Florent Pantin1, Ronan Sulpice2, Gaëlle Rolland1, Maria Piques2, Myriam Dauzat1, Mark Stitt2, Yves Gibon2, Bertrand Muller1

1INRA, LEPSE, Montpellier, France, 2MPI-MP, Golm, Germany

Understanding what limits plant growth under water deficit is an important issue because it could help in identifying key processes at which genetic variation of stress tolerance relies on, and provide prospects for breeding. We evaluated responses to soil water deficit in C metabolism and leaf growth in Arabidopsis using two complementary approaches. First, we questioned whether water deficit depreciates or improves the C status of the plant. Most activities of enzymes from central metabolism were

increased under water deficit, while all C metabolites accumulated. Strikingly, only glutamate dehydrogenase and acid invertase activities, which are known to increase under sugar starvation, decreased under water deficit. A survey of 20 sugar responsive genes then confirmed that in WT, carbon status was improved by water deficit. Furthermore, it showed that C status was improved at night in the starchless mutant *pgm*, which experiences C starvation every night under well watered conditions. Second, we evaluated the impact of C metabolism on the response of leaf growth to water deficit by using a set of mutants affected in starch metabolism. During the early steps following the emergence of a leaf, strong day-night growth fluctuations were visible in these mutants only, in phase with the fluctuations of their C status. Moreover, leaf growth at night in these mutants was increased by water deficit as compared to well watered conditions. Finally, the extent of the daily accumulation of transient C forms was strongly related to the rate of leaf growth at night, and this association became looser as the level of water deficit increased.

Taken together, these findings suggest that C metabolism has a strong impact on growth in the early, heterotrophic, stages of the leaf, but that this influence decreases with leaf age and water deficit, probably as other limitations take place.

P229 Two novel proteins, POPEYE and BRUTUS, play opposing roles in regulating root responses to iron deficiency

Terri Long, Jean Wang, Philip Benfey

Duke University, Durham, NC, USA

Understanding the dynamics of iron absorption in plants and the regulation of plant responses to iron deficient conditions is essential for developing plants with increased tolerance to nutrient-poor soils and elevated nutritional content. In a previous study we generated a high-resolution transcriptional profile of the Arabidopsis root in response to iron deficiency. Our results showed that hundreds of genes, including those with a known role in iron uptake and homeostasis, are transcriptionally activated or repressed in roots within 72 hours of exposure to iron deficient growth conditions, in specific cells and developmental zones. From these datasets we have identified two novel regulators of the iron deficiency response, *POPEYE (PYE),* and *BRUTUS (BTS)*. We determined that *PYE* and *BTS* expression are induced in the root vasculature by iron deficiency. Notably, the vasculature is the most responsive region of the root according to our microarray analyses, and the site of expression of many genes known to play a role in iron homeostasis. We also provide evidence that both PYE and BTS proteins are localized throughout the root, and that they may interact indirectly to regulate expression of subcellularly localized metal ion transporters. We propose that these two novel proteins act in an opposing manner to control subcellular iron transport under iron deficient conditions.

P230 Hypoxia responsive ERF transcription factors involved in low oxygen signalling in *Arabidopsis thaliana*

Francesco Licausi1, Joost T van Dongen2, Beatrice Giuntoli3, Antonietta Santaniello1, Peter Geigenberger2, Pierdomenico Perata1

1Scuola Superiore Sant'Anna, Pisa, Italy, 2Max Planck Institute of Molecular Plant Physiology, Golm, Germany, 3Università di Pisa, Pisa, Italy

Plants often experience challenging hypoxic conditions because of soil waterlogging or complete flooding. Previous microarray analyses revealed that members of the Ethylene Responsive Factor family (ERF) are upregulated in different plants by oxygen deficiency. In the present work, we investigated the role of two hypoxia-responsive ERF (HRE1 and HRE2) in low oxygen signalling and tolerance in *Arabidopsis thaliana*.

The expression pattern of the genes encoding these two transcription factors was analysed by means of RealTime PCR. Homozygous knock-out mutants and overexpressing lines for both transcription factors were obtained and compared with the wild-type with respect to their tolerance to anoxic treatments, fermentative enzyme activities and expression of typical anaerobic genes.

Expression of *HRE1* was observed to be stimulated by low oxygen only in root tissues, with a transient pattern, whereas *HRE2* responds to oxygen deficiency in all cell types and maintains its expression constant for several hours. Moreover *HRE1*, but not *HRE2*, requires protein synthesis to be upregulated, since treatments with translation inhibitors prevented its anaerobic induction. Transgenic Arabidopsis plants overexpressing *HRE1* showed an improved tolerance to anoxic treatments whereas a double knock-out mutant was more sensitive than the wild-type. Molecular and biochemical analyses mirrored this trend as they showed a general increased expression of the anaerobic genes together with an increase in fermentative activities under hypoxic conditions, but not in normoxia.

In conclusion, we hypothesize that the two characterized ERF transcription factors are necessary, but not sufficient, for the low oxygen response in *A. thaliana.*

P231 *OPEN STOMATA 3,* an ABC transporter implicated in ABA signalling, drought and light response

Aleksandra Wasilewska1, Nathalie Leonhardt2, Marta Riera3, Christiane Valon1, Jérôme Giraudat1, Sylvain Merlot1, Michael R Blatt4, Jens Uwe Sutter5, Jeffrey Leung1

1Institut des Sciences du Vegetal, CNRS, Gif-sur-Yvette, France, 2CEA Cadarache, CEA-CNRS, St Paul les Durance, France, 3Consorcio CSIC-IRTA, Barcelona, Spain, 4University of Glasgow, Glasgow, UK, 5University of Strathclyde, Glasgow, UK

Mutants sensitive to progressive water deficit are characterized by excessive transpiration due to the failure of stomatal closure and can therefore be detected as cold plants by infrared imaging. Among the signalling mutants,

three are collectively named *open stomata (ost)*. The corresponding *OST1* and *OST2* genes encode an ABA-activated kinase and a proton ATPase, respectively.

The current work deals with *OST3*, which encodes an ATP-binding cassette (ABC) transporter. There are over 120 members of the ABC superfamily in *Arabidopsis thaliana*. Most of them are membrane-bound proteins that transport a diverse range of substances across the phospholipid bilayer.

Characterisation of the mutant phenotype confirmed that *ost3* transpires excessively. We have also shown that the *ost3* mutations reduce seed dormancy but seed sensitivity to exogenous ABA seems unaffected. The guard cells of *ost3* are impaired in responses to ABA and light, but are normal with respect to low level of CO2 which stimulates stomatal opening.

OST3 is expressed mainly in leaves, particularly in guard cells, but it is low in root tissues. Transgenic expression of the OST3 protein fused to GFP in the *ost3* mutant can rescue the phenotype and moreover, the fusion protein is targeted exclusively to the plasma membrane suggesting that it has a role in intercellular transport required for ABA signal perception.

Using the Split Ubiquitin yeast system and *in planta* BiFC assay, we found that OST3 interacts with the OST1 protein kinase. The observation is also consistent with the fact that OST3 can be phosphorylated by OST1 *in vitro*. Furthermore, a more than additive, severe phenotype of the double mutant suggests a synergestic action of OST1 and OST3. Therefore we suggest that the two proteins identified by our genetic screen may function in the same signalling complex in mediating stomatal response.

P232 Phosphatidic acid, a lipid second messenger involved in early abiotic stress signaling

Fionn McLoughlin1, Henk L Dekker1, Magdalena M Julkowska1, Dieuwertje van der Does1, Chris G de Koster1, Christiane Laurière2, Grazyna Dobrowolska3, Michel A Haring1, Teun Munnik1, Christa Testerink1

1University of Amsterdam, The Netherlands, 2ISV, France, 3IBB, Poland

Lipid second messengers are typically produced upon various stimuli, functioning as docking sites for proteins. In plants, phosphatidic acid (PA) accumulates in response to biotic and abiotic stress stimuli, including drought, salinity, cold, wounding and pathogen infection. The increase in PA occurs rapidly and might therefore be involved particularly in early responses. How PA exerts its effects is largely unclear, due to the lack of characterized target proteins. Here, we 1) characterized two known PA targets that are involved in salt stress signalling and 2) set-up a novel proteomic approach to isolate new PA targets.

From our previous work, two members of the SNF1-related protein kinase (SnRK2) family were identified as PA binding proteins, i.e. SnRK2.4 and 2.10. The SnRK2 subfamily is plant specific and most family members have been shown to be activated by salt in protoplasts. Using hydroponically grown *A. thaliana* plants expressing GFP-SnRK fusions, we found induction of SnRK2.4/2.10 activation within minutes after exposure to salt. Knock-out mutants of both isoforms, as well as a double mutant, have been isolated and are currently being characterized with respect to salt tolerance. The PA-binding region of SnRK2.4 was identified, which will allows us to pinpoint the effect of PA-binding on its activation - and *in vivo* function.

To find novel PA targets, peripheral membrane proteins were isolated from control and salt-stimulated A. thaliana cell cultures. PA-binding proteins from these fractions were isolated with PA affinity beads and subsequently identified by mass spectrometry. Proteins that are enriched in the stimulated sample are promising candidates, which will be further analyzed with respect to lipid binding specificity and function.

P233 The tonoplast nitrate transporter AtCLCa is involved in stomata movement and its activity is regulated by phosphorylation

S Wege1, S Filleur1,2, A De Angeli3, S Thomine1, F Gambale3, H Barbier-Brygoo1

1Institut des Sciences du Végétal, CNRS UPR 2355, 1 Avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France, 2Université Paris 7-Diderot, UFR Sciences du Vivant, 75013, Paris, France, 3Istituto di Biofisica, CNR, Via De Marini 6, 16149 Genova, Italy

The *Arabidopsis thaliana* CLCa belongs to the ChLoride Channel (CLC) family of anion transport proteins. Unlike the mammalian members of the family, AtCLCa is most selective for nitrate and not for chloride. It mediates the accumulation of nitrate into the vacuole by a $NO3^-/H^+$ exchanger mechanism.

GUS expression studies show that AtCLCa is strongly expressed in stomata guard cells.

Furthermore, the phenotype analysis of *clca1* and *clca2* ko mutant demonstrate a role of the tonoplast NO3- transporter AtCLCa in stomata movement. We were then interested in how AtCLCa activity is regulated during stomata opening or closure. We identified a possible regulatory protein partner: a kinase, which is strongly expressed in guard cells and is activated in response to ABA. AtCLCa contains the preferred phosphorylation recognition site in its cytoplasmic N-terminus. We already confirmed the putative interaction in *in vitro* phosphorylation assays and are currently confirming the interaction *in vivo*.

Moreover, we used the patch-clamp technique on vacuoles to demonstrate a direct influence of this kinase on the activity of AtCLCa. The effect of the phoshorylation is abolished if the threonine in the phosphorylation recognition site is replaced by an alanine.

For the first time, the effect of a kinase on a CLC transporter in plants is enlightened.

And we will present some indications that this interaction is of physiological importance in stomata guard cells: it connects the ABA signalling network with the activity of a tonoplast anion transporter.

P234 QTLs underlying the response of leaf expansion to drought in *Arabidopsis thaliana* highlight different processes by which leaf area can be maintained or increased

Sébasttien Tisné1, Denis Vile1, Matthieu Reymond2, Myriam Dauzat1, Marjorie Pervent1, Christine Granier1
1INRA, 2MPI

Understanding the physiological and genetic bases of plant performance under drought is an important challenge in the context of global climate change.

Changes in leaf area caused by drought was analysed in a population of recombinant inbred lines derived from a cross between two *Arabidopsis thaliana* accessions, Ler and An-1, at two day-lengths. Quantitative trait loci (QTLs) controlling responses of leaf production and expansion to drought were identified and characterised by developmental and cellular processes.

A set of QTLs that conferred a maintain or an increase in leaf area in response to drought was identified. A combination of 3 alleles increased both leaf production and expansion but despite a spectacular effect on the response of rosette area to drought, this pathway only functioned in short days. A QTL conferred a low reduction in leaf expansion in response to drought via a low reduction both in epidermal cell area and cell number. Additionally, two QTLs conferred a low reduction in leaf expansion but just because leaf expansion was reduced in well-watered conditions, without a specific effect of drought.

Our findings highlight the values of quantitative genetic approaches for exploring processes regulating plant responses to drought and open perspectives for genetic engineering of plant performance under drought.

P235 QTL analysis of developmental and environmental effects on mineral accumulation in Arabidopsis

Artak Ghandilyan1, Luis Barboza1, Myriam Olortegui1, Dick Vreugdenhil2, Sébasttien Tisné 3, Christine Granier3, Umit B Kutman4, Ismail Cakmak4, Henk Schat5, Matthieu Reymond6, Maarten Koornneef1, Mark G M Aarts1

1Laboratory of Genetics, Wageningen University, Wageningen, The Netherlands, 2Laboratory of Plant Physiology, Wageningen University, Wageningen, The Netherlands, 3LEPSE, INRA-SUPAGRO, Montpellier, France, 4Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul, Turkey, 5Ecology and Physiology of Plants, Vrije Universiteit Amsterdam, Amsterdam, The Netherlands, 6Max-Planck-Institute for Plant Breeding Research, Köln, Germany

Increased knowledge on the genes controlling plant micronutrient concentration is important for future improvement of crop yield and nutritional value, which indirectly will be beneficial for alleviating human and animal micronutrient deficiencies. Micronutrient accumulation in plants is a quantitative trait, generally controlled by many genes. Arabidopsis is a good model to investigate mineral accumulation variation and we determined QTLs for mineral (Ca, Fe, K, Mg, Mn and Zn) and phytate (IP6) concentrations in different organs under different conditions. Four RIL populations (Ler x Kond, x Cvi, x Eri-1, x An-1) were grown on soil and/or on hydroponic medium, and compared under optimal and drought or zinc deficiency conditions. Root, rosette and/or seed mineral/IP6 concentrations were analyzed and used to identify QTLs. This thorough analysis revealed many different (>100) QTLs, reflecting the impressive genetic variation present within Arabidopsis regarding mineral accumulation. Often different QTLs were found depending on mineral, tissue type, growth condition and abiotic stress exposure. Detailed analysis of prominent QTL at the *Cry2* and *Erecta* loci, which indicated strong effects of development on mineral accumulation.

P236 Stability of heritable stress-induced loss of gene silencing

Christina Mladek1, Olga Popova2, Marc Berlinger1, Kathrin Kiok2, Werner Aufsatz2, Claudia Jonak2, Christian Luschnig1, Marie-Theres Hauser1

1Department of Applied Genetics and Cell Biology, BOKU-University of Applied Life Sciences and Natural Resources, Vienna, Austria, 2Gregor Mendel Institute of Molecular Plant Biology, Austrian Academy of Sciences, Vienna, Austria

Stressful environments induce short-term physiological responses and genetic instabilities.[1] Recent work on the heritability of stress-induced recombination rates points to the involvement of epigenetic mechanisms (2,3). Our studies show that abiotic stressors such as extreme temperatures and UV-B irradiation induce a reactivation of a transcriptionally silenced 35S-promoter GUS transgene (TS-GUS; 4) and transposable elements. Stress-induced reactivation of the TS-GUS reporter remained detectable in two subsequent generations not exposed to stress indicating that stress leads to a heritable relaxation of epigenetic control. Reciprocal crosses show that the release of gene silencing is transmitted through both gametes and independent of the presence of the transgene. However, the reactivation of the TS-GUS reporter returned to control levels in the third generation after stress exposure, suggesting a mechanism that counteracts the stability of the stress-induced epigenetic memory.

This project is supported by GENome Research in Austria (GEN-AU) and the Austrian Science Foundation (FWF).

1 McClintock, Science 226, 792 (1984)
2 Molinier *et al*, Nature 442, 1046 (2006)
3 Boyko *et al*, Nucleic Acids Res 35, 1714 (2007)
4 Morel *et al*, Curr Biol 10, 1591 (2000)

P237 Is OST1/SnRK2.6 a physiological target of PP2Cs in ABA signaling?

Florina Vlad1, Sylvain Merlot1, Pedro L Rodriguez2, Jeffrey Leung1, Christiane Laurière1

1Institut des Sciences du Végétal, UPR 2355 CNRS, 91198 Gif sur Yvette, France, 2Instituto de Biologia Molecular y Celular de Plantas, Universidad Politecnica, CSIC, 46022 Valencia, Spain

OST1/SnRK2.6 kinase acts as positive regulator of ABA signaling, being rapidly activated through phosphorylation by ABA. In contrast, protein phosphatases 2C HAB1,

ABI1, ABI2 and PP2CA act as negative regulators of ABA signaling. Hence, *PP2C* knockout mutants are hypersensitive to ABA; conversely *ost1* recessive mutant and hypermorphic mutants *abi1-1*, *abi2-1* or *hab1G246D* expressing line have an ABA insensitive phenotype. Although several interacting proteins of PP2Cs have been identified, like OST1, MAPK6, AtHB6 or SWI3B, no physiological substrate has been yet confirmed.

We reinvestigated the ABA-dependent activation of OST1 in knockout and hypermorphic mutants of *HAB1, ABI1* and *ABI2*. In these mutant backgrounds, OST1 activation is either increased or decreased respectively, leading to the conclusion that these PP2Cs are negative regulators of OST1 activation. Taken together with the previous indication that OST1 interacts with ABI1, these data suggest that OST1 might be a direct substrate of these PP2Cs.

To facilitate the identification of PP2C substrates, we developed a phosphatase profiling strategy using a combinatorial phospho-peptide array. Our analysis reveals a broad preference pattern for HAB1, nevertheless differing from the Calf Intestinal Phosphatase. Moreover, the hypermorphic G246 to D mutation seems to increase the specificity of the wild type HAB1. Our data indicate that this profiling strategy is able to differentiate subtle substrate preference between phosphatases and could be used to define the substrate preference of other phosphatases.

We are currently using this information in a bioinformatics screen to identify putative substrates of these PP2C. Our first results will be presented at the meeting.

P238 *Arabidopsis halleri* as a model organism to study the extreme complex trait of metal hyperaccumulation

Ute Krämer1, Marc Hanikenne2, Juergen Kroymann3, Ina Talke1, Mike Haydon1, Christa Lanz4, Patrick Motte2,5, Detlef Weigel4

1BioQuant Center, University of Heidelberg, Heidelberg, Germany, 2Functional Genomics and Plant Molecular Imaging, Dpt of Life Sciences, University of Liège, Liège, Belgium, 3CNRS UMR 8079, Orsay, France, 4Max-Planck-Institute for Developmental Biology, Tuebingen, Germany, 5Center for Assistance in Technology of Microscopy, Dept of Chemistry, University of Liège, Liège, Belgium

As sessile organisms plants cannot easily evade exposure to adverse environmental conditions. *Arabidopsis halleri* is a Zn/Cd hypertolerant hyperaccumulator that is found on metal-contaminated and also on non-contaminated soils in Europe and Asia. Using a comparative functional genomics approach, we aim to identify the molecular mechanisms underlying metal hyperaccumulation and associated hypertolerance, and the genetic changes that occurred during the evolution of these extreme traits.

Employing Arabidopsis microarrays, cross-species transcriptomics identified a number of candidate genes that are more highly expressed in the Zn/Cd hypertolerant metal hyperaccumulator *Arabidopsis halleri* than in the closely related non-tolerant non-accumulator species *Arabidopsis thaliana*. To analyze candidate gene functions *A. halleri* was transformed with RNA interference constructs designed to silence selected metal homeostasis candidate genes using a newly developed transformation protocol for *A. halleri.* In a complementary approach, *A. halleri* candidate genes were introduced into *A. thaliana* to test their ability to confer metal tolerance or accumulation. The results indicated that P1B-type Zn/Cd-ATPase membrane transport proteins have a central role in both metal hyperaccumulation and hypertolerance.

In order to determine the cause of high candidate gene expression in *A. halleri*, DNA fragments containing the promoter regions of candidate genes were cloned from both *A. halleri* and *A. thaliana,* and promoter swap experiments were conducted. These experiments suggested that gene copy number expansion and *cis*-regulatory changes were of major importance in the evolution of naturally selected metal hyperaccumulation and associated hypertolerance.

Our work on metal hyperaccumulation provides insights not only into how a complex physiological trait evolved, but also into how plant metal homeostasis networks can be effectively modified for phytoremediation, phytomining and bio-fortification.[5]

1 Becher *et al* (2004) Plant J 37: 251-268
2 Weber *et al* (2004) Plant J 37: 269-281
3 Talke *et al* (2006) Plant Physiol. 142: 148-67
4 Courbot *et al* (2007) Plant Physiol. 144: 1052-1065
5 Hanikenne *et al* (2008) Nature 453: 391-5.

P239 Endocycle regulator DEL1 represses *PHR1*, a UV-induced DNA-damage repair gene

Amandine Radziejwoski, Kobe Vlieghe, Tim Lammens, Sara Maes, Marcel Jansen, Claudia Knappe, Andreas Albert, Harald Seydlitz, Werner Heller, Günther Bahnweg, Dirk Inze, Lieven De Veylder

VIB Department of Plant Systems Biology, Ghent University Technologiepark 927, 9052 Gent, Belgium

DP-E2F-like 1 (DEL1/E2Fe) was previously described as an important endocycle regulator. Here we identified a new role for *DEL1* in the regulation of DNA repair proteins by analyzing microarray data. We found out that DEL1 represses the transcription of the type II CPD-photolyase *PHR1/UVR2*, a DNA-repair gene, by binding on its promoter. Plants with reduced levels of *DEL1* showed an improvement of their repair abilities upon UV treatment compared to control plants, whereas plants overexpressing *DEL1* were performing worse. These differences in sensitivity levels showed to be due to changing levels of photolyase *PHR1*. The knock-out plants had indeed higher *PHR1* levels, while the opposite was true for the overexpressing line. Double knock-out plants of *DEL1* and *PHR1* were generated and they showed as much sensibility to UV irradiation as single *PHR1* knock out, proving that the good performance of UV-treated *DEL1*KO plants is due to the action of photolyase.

P240 Two new clock proteins, LWD1 and LWD2, regulate Arabidopsis photoperiodic flowering and circadian clock

Ying Wang1,2, Jing-Fen Wu1, Shu-Hsing Wu1

1Institute of Plant and Microbial Biology, Academia Sinica, Taipei 11529, Taiwan., 2Graduate Institute of Life Science, National Defense Medical Center and Academia Sinica, Taipei 114, Taiwan

In plants, circadian clock could control daylength-dependent developmental processes such as photoperiodic flowering. The Arabidopsis circadian clock is formed by several negative feedback loops composed of morning (ex. *CCA* and *LHY*) and evening (ex. *TOC1* and *ELF4*) oscillator genes. The identification of additional clock proteins will help to better dissect the complex nature of the circadian clock. Here we show LWD1/LWD2 are new clock proteins involved in photoperiodic flowering control. LWD1 (Light-regulated WD repeats protein 1) and LWD2 share ~90% identity in amino acid sequence. The *lwd1lwd2* double mutant has an early flowering phenotype, contributed by the significant phase shift of *CO* and, therefore, an increased expression of *FT* before dusk. Under entrainment conditions, the expression phase of oscillator (*CCA1, LHY, TOC1* and *ELF4*) genes in the photoperiod pathway shifts ~3 hr forward in the *lwd1lwd2* double mutant.

In addition, the period length of these oscillator genes is shortened in the *lwd1lwd2* double mutant under both continuous light and dark conditions. This suggests that LWD1 and LWD2 are more likely to function in close proximity to or within the clock rather than in the light input pathway. Promoter-GUS fusion studies revealed that the *LWD1/2* genes are ubiquitously expressed in various organs and throughout the life cycle. The accumulation of LWD1 protein peaks at morning, suggesting LWD1 might have function in regulating the morning genes. Our studies also revealed that LWD1 primarily localizes in the nucleus. Together with the harbouring of protein-protein interaction platform, 5 WD repeats, LWD1 protein may recruit transcription factors to assemble signaling complexes in the nucleus and to regulate morning genes.

P241 A functional role for TOC1 in abscisic acid signaling

Tommaso Legnaioli, Juan Cuevas, Paloma Mas

Centre for Research in Agricultural Genomics, Barcelona, Spain

Integration of environmental information into plant metabolic responses is achieved by both abscisic acid (ABA) signalling and the circadian clock. The interaction between both signalling pathways has been long assumed although little is known about the molecular nodes where these signalling pathways converge to translate the environmental information into a physiological response. In our study, we show that the circadian clock sets the timing of hormone function in the control of plant responses to stressful environments. Mechanistically, we demonstrate the existence of a molecular feedback loop connecting the circadian system with ABA perception under dry environments. A key clock component (TOC1, Timing of CAB expression 1) binds to the promoter of the putative ABA receptor (ABAR) and regulates its circadian expression. Treatment with ABA acutely induces *TOC1* expression and this induction sets the timing of TOC1 binding and modulates the *ABAR* circadian expression. Moreover, the gated induction of *TOC1* by ABA is abolished in ABAR RNAi plants indicating that sensitized ABA perception relies on the reciprocal regulation between *ABAR* and *TOC1* expression. Genetic studies with *TOC1* and *ABAR* over-expressing and RNAi in plants showed the circadian clock controls daylength-dependent gating of ABA perception and function is essential for cellular homeostasis under dry environments.

P242 Lipophilic components of the brown seaweed, *Ascophyllum nodosum*, enhance freezing tolerance in *Arabidopsis thaliana* by priming osmolyte accumulation and enhancing fatty acid unsaturation

Prasanth Rayorath1, Junzeng Zhang2, Christopher Kirby3, Bernhard Benkel1, Mark Hodges4, David Hiltz5, Alan Critchley5, Balakrishnan Prithiviraj1

1Department of Plant and Animal Sciences, Nova Scotia Agricultural College, Truro, NS, Canada, B2N 5E3, 2Institute for Nutrisciences and Health, National Research Council of Canada, Charlottetown, PE, Canada, C1A 4P3, 3Crops and Livestock Research Centre, Agriculture and Agri-Food Canada, 440 University Avenue, Charlottetown, Prince Edward Island C1A 4N6, 4Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, Kentville, NS, Canada, B4N 1J5, 5Acadian Seaplants Limited, 30 Brown Avenue, Dartmouth, NS, Canada, B3B 1X8.

Extracts of brown seaweed *Ascophyllum nodosum* improve freezing tolerance of plants. The bioactive lipophilic components (LPC) treatment of Arabidopsis resulted in significant increase in proline and total sugars. LPC treatment of Arabidopsis mutants *p5cs1* (defective in stress induced proline accumulation) and *sfr4* (defective in sugar accumulation) did not protect them against freezing injury suggesting the role of these metabolites in the LPC induced freezing tolerance. 1H NMR metabolite profile of LPC treated Arabidopsis plants exposed to freezing stress revealed a spectrum dominated by chemical shifts (δ) representing soluble sugars, sugar alcohols, organic acids and fatty acids as compared to control plants. Additionally, 2D NMR (COSY, TOCSY, HSQC, and HMBC) spectra suggested LPC treatment increased the degree of unsaturation of fatty acids as compared untreated plants. Global transcriptome analysis revealed that LPC treatment altered the expression of 1,113 genes compared to untreated control. Analysis of AraCyc-defined metabolic pathways revealed that a number of genes which are known to be important in the accumulation of compatible osmoprotectants and lipids were significantly affected besides antioxidant synthesis pathway. Collectively, *A. nodosum* LPC component mediates freezing tolerance in Arabidopsis by priming accumulation of osmoprotectants and by increasing the degree of unsaturation of fatty acids.

P243 Disentangling *Arabidopsis thaliana* responses to combined drought and thermal stresses

Denis Vile1, Marjorie Pervent1, Michaël Belluau1, Jean-Jacques Thioux1, Jérôme Joubès2, Christine Granier1

1Laboratory of Plant Ecophysiological responses to Environmental Stresses, UMR759, INRA-SUPAGRO, F-34060 Montpellier, France, 2Laboratoire de Biogenèse, Membranaire, UMR5200, CNRS-Université Bordeaux 2, F-33076 Bordeaux, France

Water deficit and high temperature are important environmental factors restricting plant growth and productivity in many areas in the world. The two stresses often occur simultaneously in the field but little is known about how their combination impacts plant development, physiology and productivity. Of additional concern is global climate change, which will presumably increase global temperature, increase the occurrence of high temperatures, change the distribution of precipitation, and intensify drought in many areas, leading to reduction of productivity and to biodiversity changes. This work aimed to evaluate the natural diversity of responses to isolated and combined soil water deficit and thermal stress in *A. thaliana*. Integrated functions such as whole plant growth and development, and more elementary physiological and developmental processes were analysed in full factorial experiments. Ten ecotypes were used to test to what extent their responses could be related to their ecological origin, and if this variability was related to functional traits such as leaf structure, stomatal conductance, or cuticular waxes. Mutants, affected in stomatal closure and cuticular wax production were used to evaluate the role of these specific functions. Both stresses reduced plant performance, but there is evidence for a strong genotype x environment interaction on plant physiology and metabolism. Among the processes involved, stomatal closure and reduction in transpiration fluxes in response to water deficit could in turn cause an increase in leaf temperature, thus increasing plant susceptibility to higher air temperature. Additionally, reduction in leaf surface in response to high air temperature could decrease plant water use and thus mitigate the effects of soil water deficit. On the other hand, heat stress could increase plant water loss through transpiration, and decrease root growth, thus increasing plant susceptibility to water shortage.

P244 Modulations of *AtGSTF10* expression induce stress tolerance and BAK1-mediated cell death

Sun Young Kim, Hyun Kyung Kang, Hee Young Ryu, Hyun Mi Park, Ji Young You, Beg Hab Kim, June Seung Lee, Kyoung Hee Nam

Division of Biological Science, Sookmyung Women's University, 53-12 Chungpa-dong 2ga, Yongsan-gu, Seoul 140-742, Republic of Korea

Glutathione S-transferases (GSTs) are essential proteins involved in cellular detoxification. The expression of GSTs has been studied extensively under various environmental stressors including xenobiotics. Here, we have isolated AtGST10, one of the phi classes of AtGSTs on the basis of its interaction with the BRI1-Associated Kinase 1 (BAK1) in a yeast two-hybrid screen. BAK1 is a leucine-rich-repeats containing receptor-like kinase (LRR-RLK), acting in both brassinosteroid signaling and pathogen-associated molecular patterns (PAMP)-triggered plant defense responses. We found that AtGSTF10 binds to BAK1 through its N-terminal domain. AtGSTF10 is expressed ubiquitously in plant tissues, and the endogenous transcript level of *AtGSTF10* was not induced by plant growth regulators or abiotic stressors, except drought, unlike other GSTs. Overexpression of *AtGSTF10* conferred higher tolerance to salt and disturbed redox status of transgenic plants. The down-regulation of *AtGSTF10* produced by RNA interference caused reduced tolerance to abiotic stress and an accelerated senescence of transformants, indicating that AtGSTF10 is involved in stress tolerance and the BAK1-mediated spontaneous cell death signaling pathway in Arabidopsis.

P245 Enolase, a cross-link between glycolysis and stress response

Nina Jaspert1, Katrin Weckermann1, Markus Piotrowski2, Claudia Oecking1

1ZMBP, University of Tuebingen, Tuebingen, Germany, 2Ruhr-University Bochum, Bochum, Germany

The Arabidopsis enolase LOS2 is a bifunctional protein. In addition to the enzymatic conversion of 2-phosphoglycerate to phosphoenolpyruvate in the glycolytic pathway, LOS2 was suggested to function as a transcriptional repressor in cold stress responses (Lee *et al*, 2002). Accordingly, a LOS2-GFP fusion protein is localized in the cytoplasm as well as the nucleus.

In a proteomic approach, we identified LOS2 as a putative interaction partner of 14-3-3 proteins which have been implicated in the regulation of distinct biological processes: Plant 14-3-3 proteins bind a range of transcription factors, signalling proteins as well as enzymes involved in nitrogen and carbon metabolism.

With the aim of analyzing the *in vivo* relevance of this interaction, a *los2* knock-out was identified, which shows a severe dwarfish phenotype. This is intriguing taking into account that Arabidopsis expresses two further enolases (ENO1, ENO2). ENO1 is exclusively localized to plastids, while ENO2 shows a subcellular localization comparable to LOS2. In contrast to LOS2, the recombinant ENO2 shows no glycolytic activity *in vitro*, which may explain the severe *los2* phenotype. In order to analyze the bifunctionality of LOS2 in more detail, the knock-out line was transformed with LOS2 fused to a nuclear export signal (NES). This rescues the dwarfish *los2* phenotype. However, the plants are hyposensitive to salt and osmotic stress, suggesting that nuclear localized LOS2 is involved in such stress response. Interestingly, BiFC-analysis indicates that the interaction between 14-3-3 and LOS2 is restricted to the cytoplasm, supposing that 14-3-3 proteins might regulate LOS2 mediated stress responses by sequestering LOS2 in the cytoplasmic compartment. A transformation of the *los2* knock-out line with the wildtype versus a non 14-3-3

interacting mutant variant of LOS2 could prove this hypothesis. Hence we will focus our work on the identification of the 14-3-3 binding motif.

P246 Functional Analysis of ARR4 in different subcellular compartments

Janika Witthöft, Virtudes Mira-Rodado, Klaus Harter
ZMBP, Tübingen, Germany

The Two Component System is a signal transduction mechanism that, by the means of a phosphorelay, allows the plant to respond to environmental stimuli (Stock *et al*, 2000). The Arabidopsis Response Regulator 4 (ARR4) acts as the output element of a two-component signalling pathway that is most probably triggered by cytokinin (Mira-Rodado *et al*, 2007). After activation by phosphorylation, ARR4 interacts with the red/far-red light photoreceptor phytochrome B (PhyB) leading to the stabilization of its active form which results in enhanced photomorphogenic responses (Sweere *et al*, 2001; Mira-Rodado *et al*, 2007). Mutation of the phosphorylatable aspartate to asparagine within the receiver domain creates a version of ARR4 that negatively affects photomorphogenesis (Mira-Rodado *et al*, 2007).

Due to the stabilization of active Phytochrome B, ARR4 represents a link between the two-component system and red light signalling. Although the cross-talk between both pathways is well understood it is unclear in which subcellular compartment the phosphorylation of ARR4 and its interaction with PhyB take place since both proteins are localized in the cytoplasm and the nucleus.

To further investigate these questions, transgenic Arabidopsis lines have been created overexpressing either wildtype ARR4, ARR4 fused to a nuclear localisation signal (NLS) or ARR4 fused to a nuclear export signal (NES). Initial results will be presented and discussed.

1 Mira-Rodado, V., Sweere, U., Grefen, C., Kunkel, T., Fejes, E., Nagy, F., Schäfer, E. and Harter, K. (2007). Functional cross-talk between two-component and phytochrome B signal transduction in Arabidopsis. Journal of Experimental Botany 58, 2595-2607
2 Stock, A.M., Robinson, V.L. and Goudreau, P.N. (2000). Two-Component Signal Transduction. Annu. Rev. Biochem 69, 183-215
3 Sweere, U., Eichenberg, K., Lohrmann, J., Mira-Rodado, V., Bäurle, I., Kudla, J., Nagy, F., Schäfer, E. and Harter, K. (2001). Interaction of the response regulator ARR4 with Phytochrome B in modulating red light signalling. Science 294, 1108-1111

P247 Characterization of *Arabidopsis thaliana* condensin II mutants in B toxicity

Takuya Sakamoto1, Yayoi T Inui1, Toru Fujiwara1,2
1Biotechnology Research Center, University of Tokyo, Tokyo, Japan, 2CREST, JST, Japan

Excess boron (B) is toxic to plants. Although physiological and biochemical effects of B toxicity are well documented, understandings of molecular mechanisms of B toxicity is limited. To elucidate the mechanisms, seven *Arabidopsis thaliana* mutants, which are highly sensitive to excess B (3 mM boric acid), were isolated and characterized. As a result of gene mapping, we found that two of condensin II complex regulatory subunits, *CAP-H2* and *CAP-G2*, are causal genes of the mutants. These two mutants were suggested to be highly specific to excess B, because they grew similarly to wild type under other abiotic stress conditions such as salt, As and Cd excess.

In plants, another type of condensin complex, condensin I, is known. Two complexes share the same subunits in the core and have distinct regulatory subunits. After excess B treatment, transcript accumulations of all regulatory subunit genes of both types of condensins were elevated in wild type, suggesting the possible involvement of both condensins in B toxicity/tolerance. Condensins play the role for mitotic chromosome assembly and segregation. Condensins are also suggested to play roles in regulation of transcription and damaged-DNA repair in yeast and vertebrate cells. *A. thaliana* condensin II mutants were not sensitive to DNA-damaging agents, suggesting that excess B is unlikely to cause DNA damage. In root tips of condensin II mutants, abnormal cell alignments and enlarged epidermal cells were evident in excess B condition, but not in the normal condition. Expression analysis of cell cycle related genes revealed that transcripts of *CycA2;1*, which is suggested to control endoreduplication, was upregulated by excess B treatments in condensin II mutants, but not in wild type.

These results indicate the involvement of condensin II in normal cell division under B toxicity. Further analysis with BY-2 cells showed that excess B causes cell cycle arrest at prophase and changes chromosome structure abnormal. Additional analysis using RNAi technique of condensins in BY-2 cells is expected to provide us with new information about condensins function in B toxicity/tolerance.

P248 Nitrogen-regulated transcription factor, AGL21, controls lateral root development in Arabidopsis

Akinori Suzuki1, Mayu Miyamoto1, Brian G Forde2, Hideki Takahashi1,3
1RIKEN Plant Science Center, Yokohama, Japan, 2Lancaster Environment Center, Department of Biological Science, Lancaster University, Lancaster, UK, 3Kihara Institute for Biological Research, Yokohama City University, Yokohama, Japan

The plasticity of root architecture is crucial for adaptation to limiting or excessive supplies of nutrients. Nitrogen is one of the macro-nutrients required for plant growth and can modify plant root system architecture, which is highly responsive to nitrate. When roots encounter localized supply of nitrate, lateral root elongation is stimulated. Previous studies with Arabidopsis have demonstrated a MADS box transcription factor, ANR1, as a positive regulator for this elongation step. However, the regulatory mechanisms that control root development under low-nitrogen environment are mostly uncharacterized. In this study, we found that AGL21, an ANR1 homologue, is required for the growth of lateral roots when supply of nitrogen is limited. The lengths of lateral roots were

shorter in *agl21* mutants than in the wild-type plants under low-nitrate conditions. By contrast, both the numbers and lengths of lateral roots increased by over-expression of *AGL21*. On the other hand, the lateral root lengths were not significantly different between *anr1* mutants and wild-type plants, and also between *agl21anr1* and *agl21* mutants under low-nitrate conditions, indicating that *anr1* mutations may cause no apparent defects in lateral root development nor may act additively to *agl21*. Transgenic plants harbouring *AGL21* promoter-GFP fusion gene indicated that *AGL21* is expressed in epidermis, columella and lateral root cap cells of the meristematic region of lateral roots under low-nitrate conditions. These results suggested that AGL21 is an essential factor to sustain lateral root elongation under low-nitrate conditions and that this regulatory pathway is independent of the ANR1-mediated mechanism which responds to localized supply of nitrate.

P249 Investigation of genetic influence on the responses of Arabidopsis to environmental change

Tebogo Selebatso

University of Sheffield, Sheffield, UK

Increase in global carbon dioxide and temperature are characteristic features of global climatic change. There are overwhelming reports on the future increase of these environmental factors by IPCC, (2007). How plants respond to these changes has important implications for the performance of both natural and agricultural systems.

Ecotypes originating from different altitudes will have evolved under different environmental conditions. The aim of this project is to quantify the effects of environment (carbon dioxide concentration and temperature) on anatomical features, phenology and plant fitness of ecotypes of *Arabidopsis thaliana* originating from different geographical areas and different altitudes. Eleven ecotypes with altitudinal origin of 0 to 1200m were grown in controlled environmental experiments to assess the extent to which genetic constraints influence phenotypic plasticity. Plants were subjected to two levels of CO_2 at 400ppm (ambient) and 800ppm (elevated) and temperature regimes of 22 and 28°C. Plants were analysed for epidermal anatomy, time to flowering and to maturity and plant fitness.

Results suggest that ecotypic origin has significant effects on responses to both elevated carbon dioxide and temperature. Such knowledge of plants responses under predicted environmental change is critical to preservation of vegetation, both natural and agricultural.

P250 Role of *Arabidopsis thaliana* stress-response genes in heavy metal tolerance

Vanessa Menezes-Oliveira, Darlan Quinta, Susana Loureiro, Paula Duque, Amadeu M V M Soares

CESAM, Universidade de Aveiro, Campus Universitario de Santiago, 3810-193, Aveiro

Heavy metals are natural elements that can be found in the environment at various concentrations due to the basal levels that can be found in the bedrock. However, they can become a problem when the concentrations available in the environment are increased by human activities causing detrimental effects to plants and animals. In plants there is a homeostatic mechanism that maintains a suitable concentration of essential metallic ions in their tissues. Adaptation and tolerance are different key processes that enable plants to survive when stressor levels are increased. Such key roles may be played by genes involved in the response to environmental stress. To understand these response patterns, several loss of function mutants for membrane transporters and stress-response genes have been generated to investigate their role in heavy metal (lead) detoxifications in the model plant *Arabidopsis thaliana*. Thus, ecotoxicological tests were carried out (ISO 11269-2 protocol adaptations) using these knockout plants to confirm the importance and role of the corresponding genes in the stress response process. The direct influence of Pb on the emergence, biomass and growth of *Arabidopsis thaliana* plants was evaluated and compared between wild-type and mutants.

P251 Protein phosphorylation and a 14-3-3 protein binding in Arabidopsis guard cells in response to ABA

Yohei Takahashi1, Yuta Ebisu1, Toshinori Kinoshita2, Ken-ichiro Shimazaki1

1Kyushu University, Fukuoka, Japan, 2Nagoya University, Aich, Japan

Under drought, abscisic acid (ABA) promotes stomatal closure to prevent water loss. Although protein phosphorylation plays an important role in ABA signaling, little is known about this process in biochemical levels. Previously, we searched the substrates of protein kinases involved in ABA signaling through the binding of a 14-3-3 protein to the proteins that are phosphorylated using Vicia guard cell protoplasts. We found that a protein of 61 kDa was phosphorylated rapidly in response to ABA in a guard cell specific manner. In this study, we used the guard cell protoplasts from Arabidopsis and found 53 and 43 kDa proteins were bound by a 14-3-3 protein in response to ABA. We characterized the phosphorylation of these proteins and determined the location of the proteins in ABA signaling pathways using several mutants including *abi1-1*, *abi2-1* and *srk2e/ost1*. The ABA-induced 14-3-3 protein bindings to the proteins were impaired significantly in *abi1-1* and *abi2-1* mutant plants, but, the *srk2e/ost1* mutation did not affect the bindings. Furthermore, hydrogen peroxide, a second messenger for ABA-induced stomatal closure, did not elicit these responses. From these observations, we localized the 53 and 43 kDa proteins in ABA signaling pathways in Arabidopsis guard cells.

P252 Arabidopsis activation tag lines in studying Zn efficiency

Gokhan Hacisalihoglu

Florida A&M University, Tallahassee, FL 32307 USA

Zn is an essential nutrient for all living organisms. Understanding how plants respond to low Zn is important, as

Zn deficiency is a major factor affecting crop productivity throughout the world. Zn efficiency (ZE) is the ability of plants to maintain high yield under low-Zn conditions. The objective of this study was to perform large-scale screening of 62,000 T-DNA activation tagged Arabidopsis lines to identify mutants with superior ZE. Our hypothesis was that overexpression of certain genes will lead to ZE in Arabidopsis. We have established a hydroponic screening system and isolated a number of putative Arabidopsis mutants, which we are currently screening for confirmation. The hydroponic solution contained 1 mM KNO_3, 1mM $Ca(NO_3)2$, 0.05 mM $NH_4H_2PO_4$, 0.25 mM $MgSO^4$, 0.1 mM NH_4NO_3, 50 uM KCl, 12.5 uM H_3BO_3, 0.1 uM H_2MoO_4, 0.1 uM $NiSO_4$, 0.4 uM $MnSO^4$, 1.6 uM $CuSO_4$, 96uM $Fe(NO_3)$ 3, 118 uM H_3HEDTA, and 2 mM MES at pH 6.0. Zn-efficient Arabidopsis mutants will be recovered and moved to agar plates with full nutrients for 3 days and to soil. After growing the mutants in soil until flowering and seed set, T3 seeds will be harvested for secondary screening. In summary, these findings will allow us in future work to isolate gene(s) that affect ZE in Arabidopsis. This project has also broader impacts including training of minority students in plant biology. Current progress in this study and further results in support of our hypothesis will be presented.

P253 Characterization of a CNG channel subfamily in *Arabidopsis thaliana*

Annette Kugler1, Barbara Köhler2, Petra Dietrich1

1University of Erlangen, Molecular Plant Physiology, Staudtstr.5, 91058 Erlangen, Germany, 2University of Potsdam, Max-Planck-Institute for Molecular Plant Physiology Golm, Am Muehlenberg1, 14476 Potsdam, Germany

In *Arabidopsis thaliana*, the family of cyclic nucleotide gated channels (CNGCs) is composed of 20 members. Previous studies indicate that plant CNGCs are involved in cellular cation homeostasis, growth processes as well as responses to abiotic and biotic stresses. Here, we studied the expression patterns and regulation of two CNG channels, *AtCNGC19* and *AtCNGC20*, which constitute one of the five subfamilies in Arabidopsis. GUS, GFP and luciferase reporter assays revealed the spatial and developmental expression patterns. While *CNGC19* was already expressed in roots of younger plants, the activity of the *CNGC20* gene increased during development and was maximal in mature and senescent leaves. *CNGC19* expression was restricted to the vasculature while *CNGC20* accumulated in mesophyll cells surrounding the veins, and was also found in epidermal and guard cells. Interestingly, the activity of both genes was increased upon salt stress: In the shoot, both genes were upregulated in the presence of elevated NaCl, but not mannitol concentrations. For *CNGC20*, salt induction occurred with similar kinetics irrespective of whether shoot or root were exposed to salinity. In the root, *CNGC19* did not respond to changes in salt concentration in a time window that induced gene activity in the shoot. Homozygous T-DNA insertion lines for *CNGC19* and *CNGC20* developed a growth phenotype similar to that of the wild type in the presence of up to 75 mM NaCl, and no differences in total Na, K, Ca or Mg ion contents of the shoots were measured, respectively. Together, the results strongly suggest that both channels are involved in response to salinity of different cell types in the shoot. The loss of either *CNGC19* or *CNGC20* alone, however does not lead to an altered growth response at elevated salt concentrations.

P254 Analysis of the Arabidopsis *AtMYB60* promoter, specific for guard cell expression

Eleonora Cominelli, Alessandra Albertini, Priscilla Francia, Massimo Galbiati, Chiara Tonelli

Department of Biomolecular Sciences and Biotechnology, University of Milan, Italy

To improve plant performance under water stress conditions a useful strategy consists in manipulating gene function specifically in guard cells. Therefore the isolation of guard cell specific promoters is an important goal.

We previously reported that *AtMYB60* is a R2-R3 MYB transcription factor of Arabidopsis, expressed in guard cells and directly involved in the regulation of stomatal movements. *AtMYB60* promoter drives specific reporter gene expression in guard cells.

Here we report the deletion analysis of *AtMYB60* promoter through transgenic lines harbouring different portions fused to the *AtMYB60* reporter gene. We identified the minimal promoter fragment necessary for *AtMYB60* gene expression in guard cells. Through site specific mutagenesis we identified DNA binding sites with a role in the regulation of *AtMYB60* promoter activity.

AtMYB60 promoter drives specific reporter gene expression in guard cells also in tobacco and in tomato transgenic plants, indicating a general mechanism for stomatal gene regulation in different species. Therefore *AtMYB60* promoter could be considered as a useful instrument for the modulation of gene expression in guard cells.

P255 Overlapping function of ZTL, LKP2 and FKF1 in the Arabidopsis circadian clock

Antoine Baudry1, Takatoshi Kiba3, Sheen Lu4, Nam-Hai Chua3, Elaine M Tobin4, Steve A Kay1, Takato Imaizumi2

1Section of Cell and Developmental Biology, Division of Biological Sciences, University of California, San Diego USA, 2Department of Biology University of Washington, Seattle USA, 3Laboratory of Plant Molecular Biology, The Rockefeller University, New York USA, 4Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles USA

The circadian system controls the duration and timing of various biological processes that oscillate with a 24-h rhythm. In Arabidopsis, many clock-associated genes have been already identified. One of the important components for clock progression is ZEITLUPE (ZTL). ZTL is a 26S proteasome-associated F-box protein that possesses a light-absorbing LOV domain. ZTL is involved in the control of the circadian period at least by targeting two related clock-associated proteins, TIMING OF CAB EXPRESSION1 (TOC1) and PSEUDORESPONSE REGULATOR5 (PRR5), for proteasome-dependent degradation.

There are two ZTL-related proteins named LOV KELCH PROTEIN 2(LKP2) and FLAVIN-BINDING KELCH REPEAT F-BOX1 (FKF1) in Arabidopsis. FKF1 is involved in photoperiodic flowering time regulation. FKF1 degrades a transcriptional repressor of *CONSTANS (CO)* named CYCLING DOF FACTOR 1 (CDF1). ZTL, LKP2 and FKF1 share more than 70% of their amino acid sequence identities throughout the proteins; however the molecular function of both LKP2 and FKF1 in clock regulation was not well documented.

We obtained results that indicate potential roles of LKP2 and FKF1 in the circadian clock by analyzing a combination of mutations for all three genes. The *fkf1* mutation enhances the phenotype of the *ztl* mutant, but we could not detect any additive phenotype in the *ztl lkp2* double mutant. We also found that the amplitude of certain clock genes expressed in the morning is decreased in the *ztl* multiple mutant background. To explain the phenotype we analyzed the stability of TOC1 and PRR5 proteins. We found that both TOC1 and PRR5 were more stable in the mutant plants than in the wild type plants. Based on these findings, we will discuss the molecular mechanisms by which the *ZTL* family genes regulate Arabidopsis circadian clock systems.

P256 Evidence for gravity-induced calcium response in Arabidopsis under microgravity condition

Masatsugu Toyota1,2, Takuya Furuichi1,3, Hitoshi Tatsumi1, Masahiro Sokabe1,4,5

1Department of Physiology, Nagoya University Graduate School of Medicine, Japan, 2Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan, 3Group of Plant Growth Regulation, Research Institute for Bioresources, Okayama University, Japan, 4ICORP/SORST, Cell Mechanosensing Project, JST, 5Department of Molecular Physiology, National Institute for Physiological Sciences, Japan

Changes in the gravity vector (gravistimulation) are known to induce an increase in cytoplasmic free calcium concentration having two peaks in *Arabidopsis thaliana* seedlings. However, it is difficult to determine whether the calcium peaks are caused by gravistimulation and/or rotation itself, since gravistimulation is generally accompanied by the rotation of specimen in ground experiments. Here, we show that the second calcium peak is purely induced by gravistimulation by separating the stimulus into rotatory and gravity ones under a 20 sec microgravity condition (μg) created by parabolic flight. When Arabidopsis seedlings were turned 180° during μg, the first calcium peak was observed immediately after a 180°-rotation even without gravity, whereas the second one was not. After the gravitational acceleration was returned to ca. 1.5g from μg, calcium concentration started to increase, producing a calcium peak corresponding to the second calcium peak. This indicates that the second calcium peak is caused by gravistimulation but not rotation. To support this, when the seedlings were turned 180° and immediately returned back to 0° during μg, only the first calcium peak could be observed probably because orientation of the seedlings was not changed before and after μg. Furthermore the amplitude of second calcium peak was linearly dependent on gravitational acceleration, suggesting that plants evolved on Earth are capable of transducing a wide range of gravity changes into calcium changes.

P257 OST1 kinase controls ABA dependant gene expression in stomata via the phosphorylation of conserved sites in ABF/AREB transcription factors

Caroline Sirichandra1, Marlène Davanture2, Benoit Valot2, Michel Zivy2, Benjamin Turk3, Jeffrey Leung1, Sylvain Merlot1

1CNRS, Institut des Sciences du Végétal, 1 avenue de la Terrasse, Bât. 23, 91198 Gif-sur-Yvette, France, 2UMR de Génétique Végétale, INRA/Université Paris XI/CNRS/INA PG, Ferme du Moulon 91190 Gif-sur-Yvette, France, 3Department of Pharmacology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520, USA

In response to water stress/dehydration, the Arabidopsis kinase OST1 relays the abscisic acid (ABA) signal to mediate stomatal closure and reprogramme gene expression. By a combined biochemical screen and bioinformatics, we have identified several plasma membrane-resident transporters and transcription factors among the putative OST1 phosphorylation targets. In particular, this screen reveals several sites in the ABA-dependent ABF/AREB b-zip transcription factors that are quasi-optimal sites for OST1.

We focused our study on ABF3, expressed in stomata like OST1. We first obtained genetic evidence that both *OST1* and *ABF3* are implicated in the ABA dependant expression of genes in stomata and confirmed the phosphorylation of the predicted sites by OST1 *in vitro*. BiFC experiments also indicated that OST1 and ABF3 proteins interacted in the nucleus of guard cells supporting that ABF3 is a genuine physiological substrate of OST1. Moreover, we obtained molecular evidence that OST1 phosphorylates ABF3 *in vivo* to stabilize the transcription factor.

The direct phosphorylation of ABF transcription factors by OST1 acting early in ABA signaling also suggests a straightforward architecture of ABA signaling after the activation of OST1 to regulate gene expression.

P258 Physical interaction between a pseudo-response regulator and a calmodulin-like protein involved in abiotic stress and ABA responses

Alexandre Perochon, Stefan Dieterle, Didier Aldon, Jean-Philippe Galaud, Martine Charpenteau, Benoit Ranty

UMR 5546 CNRS / Universite Toulouse 3

Besides calmodulin (CaM), an ubiquitous calcium sensor in eukaryotes, plants possess a unique set of calmodulin-like proteins (CMLs) that may function as calcium sensors thus extending the involvement of CaM family in cell signalling. We previously reported that CML9 plays a critical role in abiotic stress tolerance and ABA responses in

Arabidopsis (Magnan *et al* 2008 Plant J. 56: 575). Disruption of CML9 in insertional mutants confers an enhanced tolerance to drought and salt stress correlated to an ABA-hypersensitive phenotype. To better understand the function of CML9, we searched for CML9 binding proteins by yeast two hybrid screen, which allowed us to identify a two-component pseudo-response regulator (PRR). The two-component system is a major signalling mechanism, known to mediate plant responses to various stimuli such as hormones, light and stress. The PRR protein identified here consists in a receiver-like domain at the N terminus followed by a GARP DNA-binding motif, a proline rich region and a GCT box. Its biological function is still unknown. We observed that the PRR specifically interacts with CML9 and CML8, two closely related CMLs, but not with typical CaM in yeast.

Studies on serial deletions in the PRR suggest that both domains of the protein are required for interaction with CMLs. Microscopy analysis indicates that the PRR as a fusion to a fluorescent protein localizes predominantly in the nucleus of plant cells, and FRET-FLIM measurements show the physical interaction of PRR with CML9 in the nuclear compartment. Investigations on the biochemical functions of the PRR and its potential role in ABA responses are under progress.

P259 Mapping genes involved in growth response to potassium starvation

Aina Prinzenberg1, Hugues Barbier2, Maarten Koornneef1, Matthieu Reymond1

1Max Planck Institute for Plant Breeding Research, Cologne, Germany, 2Institut de Biologie Moléculaire des Plantes du CNRS, Strasbourg, France

Availability of potassium affects plant growth and consequently acts on yield stability in crops. Although a lot is known about potassium uptake and transport, the regulatory network for potassium homeostasis remains elusive. Our aim is to identify genes involved in growth responses caused by potassium starvation. Plant growth is a multigenic, integrative trait, which varies between genotypes of one species. For that reason a Quantitative Trait Loci (QTL) analysis is an appropriate method to identify genes involved in complex traits, such as growth responses to environmental cues. We therefore used a Recombinant Inbred Line (RIL) population of *Arabidopsis thaliana* to detect QTL for growth responses to potassium starvation. The RIL population is derived from a cross between the accessions Ler (Landsberg erecta from Poland) and Kas-2 (from Kashmir). To control the potassium availability, the plants were grown in a hydroponic system with contrasted nutrient regimes. One QTL was detected on the bottom of chromosome five and was validated using selected Near Isogenic Lines (NILs). These NILs differed in 20%, in rosette weight response to potassium starvation, compared to the parental line. Fine mapping showed that two regions with additive effects underlie this QTL. No obvious candidate gene is annotated so far for one of these regions. Thus further fine mapping is pursued and will reveal a new player in potassium stress response.

P260 Light signalling pathways mediate cold acclimation in Arabidopsis

Rafael Catala1, Joaquin Medina2, Julio Salinas1

1Departamento de Biologia de Plantas, Centro de Investigaciones Biologicas, CSIC, Madrid, Spain, 2Departamento de Biotecnologia, INIA, Madrid, Spain

External signals cannot be considered in isolation when studying the adaptive responses plants have evolved to survive an ever-changing environment. In fact, plants must process and integrate all the surrounding signals to respond adequately in all facets of development to changes in environmental conditions. In the case of cold acclimation, the correct integration of low temperature and light signals is crucial to ensure the appropriate development of this adaptive response. Nonetheless, very little is known about the molecular mechanisms that control this integration and the intermediates that are involved. Previous results from our laboratory demonstrated that the expression of an Arabidopsis light-inducible gene, *CAB1*, is also regulated by low temperature. Although *CAB1* does not contain any described low temperature responsive element in its promoter, we showed that this regulation occurred at the transcriptional level. In an attempt to identify the cis element responsible for the cold-inducibility of *CAB1*, a deletion analysis of its promoter has been performed. We have defined a new low temperature responsive element that mediates the cold induction of *CAB1* and is present in the promoters of several cold-inducible genes. Interestingly, this motif has also been described as a light-responsive element, constituting, therefore, a node of interaction between light and low temperature signaling. In addition, results will be presented showing that light signaling intermediates play an important role in cold acclimation response by regulating the cold induction of different light-responsive genes that protect Arabidopsis from the oxidative stress originated by low temperature. Our data indicate that light and low temperature integrate through light signaling pathways to guarantee the proper development of cold-acclimation response.

P261 The critical role of the Arabidopsis circadian clock at high temperature

Nicola Costa, James Hartwell, Anthony Hall

University of Liverpool, UK

The circadian clock is an internal mechanism found in most eukaryotes generating a 24h rhythm. It has evolved to anticipate predictable environmental changes and therefore make the best use of resources. In *Arabidopsis thaliana* the circadian clock controls a large number of physiological traits and the expression of about 16% of *Arabidopsis* genes. A clock synchronized with the external environment was found to be important for the growth, performance and fitness of the plant. There is also evidence that plants with a clock period matched to the environment have a substantial advantage over plants with circadian periods differing from their environment (Dodd *et al* 2005).

In the present study we investigate the importance for Arabidopsis plants of having a functional clock at high temperatures. To address this question we used *CCA1ox* transgenic plants over-expressing the clock gene *CCA1* (with an arrhythmic clock) as well as mutant lines whose period is different from the standard 24h of the wild type Col-0. The performance of the plants at different temperatures and photoperiods was measured through the visible leaf area, wet/dry weight and endpoint levels of various metabolites. The results of these experiments will be presented and discussed.

P262 *TIME FOR COFFEE* sets the circadian clock at dawn by integrating metabolic signals

Alfredo Sanchez Villarreal, Seth J Davis

Max Planck Institute for Plant Breeding Research, Plant Developmental Biology Department, Cologne, Germany

The Earth's rotation causes most organisms to face daily light/dark transitions. Organisms have evolved a mechanism, the circadian clock, to predict these cyclic transitions. These clocks are oscillators that are entrained by external stimuli and have a periodicity of about 24 hours. The Arabidopsis clock is composed of a series of interconnected feedback loops. However, how light signals set the timing of this clock remains elusive. *time for coffee (tic)* is defective in dawn sensing because the pace of the clock in this mutant is incorrectly reset between late night and dawn. To expand on TIC function and to describe the mechanism for dawn perception, we performed a microarray analysis to evaluate clock entrainment, in a TIC-dependent fashion. From this, we confirmed that tic has misexpression of clock genes, and also alters the expression of diurnal genes that are not under clock control. Further analysis showed that *tic* has disrupted stress responses and metabolic processes, which include redox homeostasis, carbohydrate metabolism and stress-related hormone signaling. With the aim to understand TIC cellular function, we screened for interactors and isolated the SNF1 stress-related kinase AKIN10, which has been proposed as a master metabolic sensor. Thus, the connection of TIC to AKIN10 could define a metabolic input to the oscillator. The physiological relevance of the interaction between TIC and AKIN10 was genetically tested and found that *AKIN10* has an effect on clock period that is dependent on *TIC*. These results prompt us to propose a hypothesis of clock entrainment by metabolic signals, derived from photosynthesis and cellular energy status, through TIC in the anticipation of the oncoming new day.

P263 The role of stomatal density in governing growth and competitive interactions in relation to water stress: Experimental observations with Arabidopsis

Ángel Ferrero-Serrano, James Hartwell, Martin Mortimer

University of Liverpool, Liverpool. UK

The trade-off between CO_2 intake and loss of water in plants is regulated in the long term by changes in leaf area and in the short term by stomatal regulation. We studied the effect of stomatal density on individual plant growth, and within populations using *Arabidopsis thaliana* as a model system. The *stomatal density* and *distribution mutant 1-2* (*sdd1-2*) mutant line, which shows increased stomatal density, was compared with wild type under well watered and water limited conditions to address three questions. Firstly, does an increase in stomatal density in the mutant result in enhanced photosynthetic CO_2 fixation; secondly to what extent are different photosynthetic rates reflected in individual overall plant growth (RGR); and thirdly, does this differential growth affect plant-plant interactions? Results suggest, that despite the fact that the relative water content of above ground tissue under both water regimes remained constant, CO_2 assimilation rates were similar for both genotypes under high water conditions, but much reduced in *sdd1-2* under water limitation. Consequently, water use efficiency was significantly reduced by water stress. As a result, higher vegetative RGRs were recorded under well-watered regimes the wild type exceeding the mutant, whereas both genotypes established a similar pattern of growth under water limitation. Under this water regime, the root:shoot biomass ratio of the wild type was significantly increased, but did not alter in *sdd1-2*. Studies of intra-genotypic competition using a plant biomass-density model revealed that vegetative biomass was more sensitive to density under the high water regime in both genotypes. Contrastingly in terms of reproductive biomass, *sdd1-2* mutants under well watered conditions showed the lowest yield in comparison to other genotype-water regime combinations.

P264 Prediction of interaction networks using transcriptional co-regulation algorithms

Achim Hahn, Joachim Kilian, Dierk Wanke, Klaus Harter, Kenneth W Berendzen

Center for Plant Molecular Biology, University of Tübingen, Germany

Many cellular processes such as signaling cascades rely on protein-protein interaction events. The prediction of unknown interaction partners is, therefore, helpful for targeted reverse-genetics and engineering approaches to fill gaps in established signaling cascades and extend the apparently incomplete interaction networks. Identifying unknown interaction networks with computational methods has been attempted by generating networks from orthologous protein interactions or from co-expression using microarray data. The latter method has been commonly used to identify proteins which presumably act together in the same signaling cascade. Here, we explore this approach by examining published and predicted protein-protein interaction data within protein kinase signaling pathways and compared them to co-expression matrices. We find several examples where elements of published kinase signaling pathways are also transcriptionally co-regulated. From our data, we concluded that the expansion of interaction networks is possible using our approach. The validation of selected putative interaction modules is presented.

P265 The role of plant synaptotagmins in plasma membrane integrity and cell survival

Arnaldo L Schapire1, Alicia E del Valle1, Julio Salinas2, Victoriano Valpuesta1, Miguel A Botella1

1Departamento Biología Molecular y Bioquímica, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain, 2Departamento de Biología de Plantas, Centro de Investigaciones Biológicas-CSIC, Madrid, Spain

Calcium dependent vesicular trafficking (CDVT) is involved in many essential physiological processes in animals. Synaptotagmins, proteins containing a transmembrane domain and two C2 domains in tandem, have been identified as key players in CDVT. Plasma membrane of animal cells can rapidly reseal disrupted sites through a tightly regulated CDVT process that is dependent on Synaptotagmin VII. In fact, this process is essential for survival and defective plasma membrane repair is linked to muscular dystrophies, a diverse group of myogenic disorders characterized by the progressive loss of muscle strength and integrity.

Despite the importance of plasma membrane repair in animals, this process has not been reported in plants so far. In a screening for salt hypersensitive Arabidopsis mutants, we found that mutations in the *SYNAPTOTAGMIN1 (SYT1)* gene results in hypersensitivity to different abiotic stresses by decreasing the integrity of the plasma membrane. This result implicates CDVT as an essential uncharacterized process in plant abiotic stress tolerance. We made an exhaustive analysis of the mutant and a biochemical characterization of the SYT1 protein reporting its Ca^{2+} and phospholipid binding characteristics. The SYT1 protein shares all characteristic domains with animal synaptotagmins and is localized predominantly to the plasma membrane, an aspect that is likely to be critical for its function. We also found that the homologous *SYT3* gene has partially redundant function with *SYT1* as the double mutant shows decreased plasma membrane integrity than single mutants. Our data indicate that Ca^{2+} dependent plasma membrane repair mediated by SYT1 and SYT3 is essential for plasma membrane integrity and cell survival.

P266 Characterising the function and regulatory pathways controlling gene expression of mitochondrial stress induced proteins in Arabidopsis

Vindya Uggalla, Olivier Van Aken, Monika Murcha, Rachel Clifton, Kristina Kuehn, Chris Carrie, Estelle Giraud

ARC Centre of Excellence in Plant Energy Biology, University of Western Australia, Perth, (W.A), Australia

Plant mitochondria are important targets of oxidative stress and several processes in plant mitochondria are inhibited by stress. During stress conditions, several genes coding for mitochondrial proteins are highly responsive, however very little is known about their function and importance under stress conditions. In this study, we are analysing two stress responsive genes encoding mitochondrial proteins. One encoding *TIM17-1* (translocase of the inner mitochondrial membrane) and the other encoding a protein with an unknown function named *UPOX1* (up-regulated by oxidative stress). There are several TIMs that form pores on the inner mitochondrial membrane through which proteins can translocate into mitochondria. However, to date only *TIM17-1* has shown a change in expression in response to stress treatments, in particular high light. Quantitative RT-PCR and micro array analyses are being conducted to further understand the function within mitochondrial stress response and the expression pattern of *TIM17-1*. UPOX1 is a plant specific protein that has been shown to be highly up regulated at both the transcript and protein levels following oxidative stress. The function of UPOX1 is being elucidated using T-DNA knock-out plants, AmiRNA and over-expressing plants in addition to various proteomic assays and micro array analysis. Currently the UPOX1 protein has been localised to mitochondria using *in vivo* GFP and *in vitro* import assays. The promoter regions of *TIM17-1* and *UPOX1* are being analysed concurrently with other mitochondrial stress responsive genes to determine which *cis*-acting regulatory elements (CAREs) are functional to ultimately identify the signalling pathways that regulate stress induced mitochondrial gene expression.

P267 Dissection of the effects of light input signals to the genetic network of the circadian clock

Kirsten Knox1, John O'Neill2, Andrew Millar1,3

1Institute of Molecular Plant Sciences, University of Edinburgh, UK, 2Institute of Metabolic Science, University of Cambridge, UK, 3Centre for Systems Biology at Edinburgh, University of Edinburgh, UK

The circadian clock is an endogenous oscillatory mechanism common to most eukaryotes and responsible for driving 24 hour rhythms. Indeed, circadian rhythms are important for controlling many metabolic, physiological and behavioural processes. Plants with incorrectly timed clocks produce significantly less biomass than plants with correctly timed clocks, showing that there is a distinct selective advantage in maintaining a clock.

A defining trait of the circadian clock is the ability to become entrained in order to exactly match the local environment. Light plays an important role in the entrainment of the clock. Several of the clock components are regulated by light, which can reset the clock relative to the current photoperiod and this may control adaptation to seasonal changes in daylength.

The Arabidopsis circadian gene network responds diversely to different light inputs. Variations in timing, duration, wavelength and intensity can all produce distinct responses. Indeed different light pathways may prevail at particular times of the day.

Dissecting the light input signals and the corresponding responses in the clock gene regulatory pathways provides vital information for the parameters required to refine mathematical models of the Arabidopsis clock.

Here we present data showing that manipulation of light is a powerful yet subtle tool when combined with our LUC reporters and QPCR in the analysis of the light input signals to the Arabidopsis clock.

P268 Arabidopsis as a tool to define the role of programmed cell death in cassava deterioration

Kimberly Jones, John Beeching
University of Bath, UK

The cassava *(Manihot esculenta)* storage root is a principle source of carbohydrate in sub-Saharan Africa and has huge potential as a robust and reliable commercial product. However, this potential is severely constrained by post-harvest physiological deterioration (PPD), which renders roots unpalatable and unmarketable within 24-72 hours. Significant involvement of reactive oxygen species (ROS) and the altered expression of genes involved in programmed cell death (PCD), suggests a role of PCD in PPD, but unlike general wound responses, repair is lost. Plant PCD is comparable to animal apoptosis and anti-apoptotic genes from mammalian systems have been used to significantly increase tolerance to oxidative stress in a variety of crop species. These anti-apoptotic genes provide an attractive tool to extend the shelf life of cassava storage roots by modulating PCD. Evaluating the effects on PPD will not only provide a greater insight into the mechanisms governing deterioration and potentially delay the response but also contribute to understanding the control of plant PCD and highlight the degree of similarity to animal apoptosis.

Constructs containing the anti-apoptotic genes *BCL-2*, *BCL-XL* and *CED-9* driven by a largely root specific patatin promoter were generated via Gateway® technology and used to transform cassava and Arabidopsis. Owing to the complex transformation process and long growth cycle of cassava, Arabidopsis provides a convenient platform to study transgene function and elucidate the expected expression levels of these constructs in cassava. Monitoring the anti-PCD response to abiotic stress should indicate whether the constructs have the potential to confer resistance to oxidative-mediated PCD. In addition, two T-DNA insertion lines that contain a T-DNA insert within genes involved in ROS regulation during abiotic stress have been isolated from Arabidopsis. Knockout lines show increased sensitivity to ROS-induced stress and will be transformed with the anti-apoptotic constructs and tested for phenotype rescue. Arabidopsis is an efficient model system in which to test and evaluate the anti-PCD qualities of the constructs prior to use in a more challenging target crop.

P269 Functional characterisation of four GDSL-lipases in *Arabidopsis thaliana*

Peter Huppenberger1, Birgit Kemmerling1, Klaus Harter1, Christopher Grefen1,2
1Center for Plant Molecular Biology, University of Tübingen, Germany, 2Division of Molecular and Cellular Biology, IBLS - Plant Sciences, University of Glasgow, U.K.

Several years ago a large family of lipases, with over a hundred members, was discovered that contained an enzymatic active site different to that of other lipases. Instead of the classical GxSxG-motif in the center of the protein, GDSL-lipases display a highly conserved octapeptide (GxSxxxxG) near their N-terminus (Brick *et al*, 1995; Upton and Buckley, 1995). Another common feature among the members of this protein family is their potential apoplastic localisation, because all members of the GDSL-lipases described so far reveal either N-terminal leader sequences or signal peptides for apoplastic secretion. In addition, there is growing evidence that plant GDSL-lipases play a functional role in biotic (*At*GLIP1, *Ca*GLIP1) and abiotic (*At*LTL1) stress responses (Oh *et al*, 2005; Narajo *et al*, 2006; Hwang *et al*, 2008) possibly through lipolytic activity in the cell wall.

Here, we used a reverse-genetic approach to characterise four less-characterised GDSL-lipases, CGM1-4 (contains GDSL-motif). Transcript analysis of the CGMs revealed overlapping but distinct expression patterns in Arabidopsis that could underscore functional differences. As in GLIP1, we observed a higher susceptibility of *cgm1* loss-of-function (LOF) lines to the necrotrophic pathogen *Alternaria brassicicola*. In addition, these lines showed a weak hypersensitive response to exogenously applied brassinosteroids. In contrast, *cgm3cgm4* double LOF lines displayed retarded germination but no significant pathogen or BR-related phenotype. Mimicking drought stress by the application of mannitol further enhanced the delay of germination. The functional relationship of the apoplastic CGMs with the different signal response pathways, to which they contribute, will be discussed.

P270 The effect of transriptional adaptor proteins ADA2a, and ADA2b on plant light responses

Konstantinos Theodoropoulos, Athanasios Kaldis, Konstantinos Vlachonasios
Department of Botany, School of Biology, Faculty of Science, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Histone acetylation is correlated with increased accessibility and transcription of associated DNA. In Arabidopsis, GCN5 is a histone acetyltransferase of H3 and H2B and physically associated with ADA2a/b proteins in large complexes, such as SAGA. Herein, we investigated the role of the transcriptional adaptors ADA2a, ADA2b and GCN5 on plant light responses including, hypocotyl elongation and gene expression.

We observed that ADA2b could act as a positive regulator of both hypocotyl elongation in the dark and light-induced inhibition of hypocotyl elongation. Under red or blue light, ADA2b might function as a negative regulator of inhibition of hypocotyl elongation. Five days-old *gcn5-1* and *ada2b-1* mutants in white light displayed reduced expression of *CAB2, RBCS-1A, LHCB2, LHCB4.3* and *COR6.6*, suggesting that both proteins might regulate positively the expression of those genes. In red light, only ADA2b could positively regulate the expression of light-induced genes.

Moreover, under blue light conditions, ADA2b and GCN5 might have distinct roles by acting as positive regulators of different light-induced genes. Within 30 min of exposure to white light, *ada2b-1* mutants resulted in transient induction of *CAB2, RBCS-1A, LHCB2, LHCB4.3* suggesting that ADA2b might function as negative regulator of early induction of those genes. In contrast, ADA2a

could function as a positive regulator of early induction of *CAB2* and *RBCS-1A*.

Furthermore, the expression of early light-induced genes, such as COR6.6, was dramatically reduced in *ada2a-2*, *ada2b-1* and *gcn5-1* suggesting that ADA2a, ADA2b and GCN5 might act as activators of early light-responsive genes. Transcriptome analysis revealed that 40% of *ada2b-1* and *gcn5-1* regulated genes were also light-r egulated. ADA2b and GCN5 might work in multiple light signalling pathways, since only 33% of the ADA2b and GCN5 dependent light-regulated genes were targets of HY5. In addition, 15% of those genes were GCN5 targets and only 5% were targets of both HY5 and GCN5.

P271 Overexpression of *AtPCS1* gene affects Cd tolerance in *Arabidopsis thaliana*: Changes in intracellular Cd and root system morphology

Letizia Zanella1, Patrizia Brunetti2, Maura Cardarelli3, Sylvia Lindberg4, Luigi Sanità Di Toppi5, Maria Maddalena Altamura1, Giuseppina Falasca1

1Dipartimento di Biologia Vegetale, Sapienza Università di Roma, Rome, Italy, 2Dipartimento di Genetica e Biologia Molecolare, Sapienza Università di Roma, Rome, Italy, 3IBPM-CNR Dipartimento di Genetica e Biologia Molecolare, Rome, Italy, 4Department of Botany, Stockholm University, Stockholm, Sweden, 5Dipartimento di Biologia Evolutiva e Funzionale, Università di Parma, Parma, Italy

The biosynthesis of phytochelatins (PCs) plays a crucial role in detoxification and homeostasis of heavy metals in plants. However, heavy metal tolerance seems to be not directly correlated to the presence of PCs, at least in some plants. Our aim was to clarify the role of PCs in Cd accumulation in *A. thaliana*. We analyzed the damages induced by the exposure for 5/9 days to different $CdSO_4$ concentrations (0, 30, 60 and 80 µM) in wt and *AtPCS1* (codifying for PC synthase) overexpressing plants. Moreover, we investigated cytosolic Cd^{2+} by epifluorescence in leaf protoplasts from wt and two *PCS1* overexpressing lines, grown at the same exogenous Cd concentrations. The morpho-anatomical analysis (including total fresh weight, primary root length, number of lateral roots, histological anomalies) showed that the root system was damaged for abundant presence of root hairs with anomalous localization, early differentiation of vascular elements, epidermal and cortical cell enlargement, and root length reduction. These anomalies were related to exogenous Cd concentration and to PC content. Moreover, the more sensitive *PCS1* overexpressing lines has the higher PCs content. Cytosolic Cd^{2+} content in leaf protoplasts treated with BTC-5N fluorochrome was higher in overexpressing lines than in wt. Taken together these results suggest that PC content influences Cd tolerance and accumulation in Arabidopsis. Further confirmation will arise from the evaluation of Cd^{2+} content in leaf protoplasts using the radioactive isotope $^{109}Cd^{2+}$. The possibility that the developmental stage interferes with cadmium sensitivity in this plant is under study.

P272 Towards a function of a stress inducible glycosyltransferase using non-targeted metabolome analysis

Veronica v. Saint Paul1, Basem Kanawati2, Theresa Faus-Keßler3, Phillippe Schmitt-Kopplin2, Anton Schäffner1

1Institute of Biochemical Plant Pathology, Helmholtz Center Munich, Germany, 2Institute of Ecological Chemistry, Helmholtz Center Munich, Germany, 3Institute of Developmental Genetics, Helmholtz Center Munich, Germany

Glycosylation of small molecules involved in defence and signalling plays an important role in creating a high diversity as well as regulating the biological activity of these compounds. There exist more than a hundred different glycosyltransferases (UGTs) in *Arabidopsis thaliana*, classified in different phylogenetic groups. However, most of them are not associated to specific biological functions. *UGT87A2* is highly responsive to several biotic and abiotic cues, which suggests an important stress related role. However, no substrate or involvement in any metabolic pathway is known. Promoter-reporter (GUS/GFP) gene fusions revealed specific expression in root tips and hydathodes as well as high induction in all senescent organs. In order to identify *UGT87A2*-related metabolites, plants having altered glycosyltransferase expression were subjected to a non-targeted metabolome analysis using high-resolution FT-ICR mass spectrometry. Whereas two knockout lines did not reveal significant metabolic changes, independent over-expression lines showed several m/z peaks indicating up-regulated metabolites. The further characterisation of these compounds lead to the identification of a new metabolite in Arabidopsis. These results suggest putative roles for *UGT87A2* in antioxidative defence or cell wall biosynthesis.

P273 Characterisation of putative targets of AtMYB60, an Arabidopsis guard cell specific transcription factor

Alessandra Albertini, Massimo Galbiati, Eleonora Cominelli, Chiara Tonelli

Department of Biomolecular Sciences and Biotechnology, University of Milan, Italy

Land plants lose over 95% of their water via transpiration through stomatal pores, distributed on the surface of leaves and stems. The opening and closing of the pore is mediated by turgor-driven volume changes of two surrounding guard cells. Engineering of stomatal responses in mutant or transgenic plants represents a valuable tool to design new crops with a more sustainable water use and opens new possibilities to improve plant survival and productivity during drought. Reverse genetics screen of T-DNA mutagenised lines, allowed the identification of a null allele of the *AtMYB60* transcription factor, involved in stomatal movements. Analysis of stomatal opening in wild-type and mutant plants revealed that the *atmyb60-1* mutation results in the constitutive reduction of stomatal aperture, and thus in reduced transpirational water loss during drought. Microarray analysis of gene expression indicated that a limited number of genes are altered in the *atmyb60-1* mutant. Here we report the first steps toward

the functional characterisation of the AtMYB60 putative targets, through mutants analysis and GUS expression patterns. These approaches will allow the characterisation of new partners of the complex stomatal stress response network.

P274 Control of EIN3 stabilization upon ethylene signaling

Bureau Marina, Lamy Geneviève, Genschik Pascal, Potuschak Thomas

IBMP-CNRS UPR-2357, 12 rue du Général Zimmer, 67084 Strasbourg Cedex, France

Ethylene plays a major role in plant growth and development by influencing a wide range of complex physiological processes throughout the entire plant life cycle, from seed germination to flowering, fruit ripening, and senescence. Ethylene signaling begins with ethylene binding to and inactivating a family of ethylene receptors. In the absence of ethylene, these receptors activate CTR1 a MAPKKK that negatively regulates the pathway. After the inactivation of CTR1, EIN2 promotes ethylene responses via the downstream transcription factor EIN3 and its close homolog EIL1. EIN3 activates primary targets of the ethylene response cascade such as *ERF1*. The transcription factor EIN3 is itself regulated at a posttranslational level by the two closely related F-box proteins EBF1 and EBF2 and this regulation is of a crucial importance for most developmental and growth responses to the hormone ethylene (Potuschak *et al*, 2003; Guo and Ecker, 2003; Gagne *et al*, 2004). F-box proteins are the substrate binding components of ubiquitin ligating SCF complexes that target substrate proteins for ubiquitin-dependent proteasomal degradation (Lechner *et al*, 2004). In the absence of ethylene, the EIN3 protein is constitutively degraded in an EBF1/2 dependent manner. However in the presence of ethylene, EIN3 protein is stabilized and accumulates. Yoo *et al* (2008) recently proposed that EIN3 stability is controlled by a bifurcate MAPK signaling pathway that acts on two EIN3 phosphorylation sites on EIN3 with opposite effects on EIN3 stability. We have produced a series of EIN3 variants containing non-phophoryable and phosphomimicking residues and we are currently testing these EIN3 variants with a special focus on their interaction with EBF1 and EBF2.

P275 Mediation of plant stress responses via mRNA turnover

Justin Walley, Katayoon Dehesh

University of California, Davis, US

To maintain homeostasis in an ever-changing environment organisms have evolved mechanisms to reprogram gene expression. One mechanism that is central to overall regulation of gene expression is mRNA degradation, which is initiated by poly(A) tail shortening (deadenylation). The CCR4-CAF1 complex is the major enzyme complex that catalyzes mRNA deadenylation and is conserved among eukaryotes. However, the components and functions of this global regulatory complex have not been well characterized in plants. We identified two *CAF1-like* genes with altered transcript levels five minutes after wounding Arabidopsis leaves by microarray analysis, indicating that these genes may play a role in stress responses. Using a combination of qRT-PCR and luciferase reporter constructs we have shown that these *CAF1-like* genes respond rapidly and transiently to a range of abiotic and biotic stresses. Additionally, we have examined their role in stress tolerance. Analysis of T-DNA null mutants demonstrates that these two *CAF1-like* genes can have unique roles in mediating response to various abiotic stresses. Consistently, there is limited overlap between the transcriptional profiles of these *caf1* mutants. Further suggesting that these *CAF1* homologs have distinct functions in mediating stress tolerance in Arabidopsis, possibly due to deadenylation of unique mRNA substrates.

P276 Establishment of a novel gain-of-function resource for functional analysis of transcription factors and analysis of novel transcription factors related to hypocotyl growth under light conditions

Youichi Kondou, Takeshi Yoshizumi, Mika Kawashima, Tomoko Kuriyama, Yukako Hasegawa, Shu Muto, Nobutaka Mitsuda, Yuko Takiguchi, Masaru Ohme-Takagi, Minami Matsui

RIKEN PSC, Yokohama, Japan

We established lines for genome wide functional analysis of Arabidopsis genes by employing gain-of-function techniques, namely, activation tagging and FOX hunting system. However the isolation of genes that cause severe phenotypes such as embryonic lethal is often difficult from ectopic expressing lines driven by CaMV 35S promoter. Because transcription factors (TF) play pivotal role for regulation of various biological functions in plants, a number of TFs should act as key regulators and may induce such lethality by ectopic expression. We have, therefore, employed glucocorticoid receptor (GR)-mediated functional induction system to avoid lethality caused by overexpression of TFs. For this purpose, we cloned coding region of 1,600 TFs by Gateway technology. Using this collection, we are establishing novel lines, in which each TF fused with GR domain is independently overproduced in Arabidopsis.

We isolated a mutant that has long hypocotyl phenotype by DEX application under long day condition. The long hypocotyl phenotype has been shown to be observed under blue, red and far-red light conditions, but not under dark conditions. The gene overexpressed in this mutant encoded a novel C2H2 type zinc finger TF. The C2H2 type zinc finger proteins are one of the largest families of TFs in Arabidopsis. The transcriptional repression domain was predicted near C-terminal in this TF. We verified by *in vivo* assay that this TF and its Arabidopsis homolog acted as transcriptional repressors. These results suggest that this novel zinc finger protein function as a negative regulator in light signal transduction and overproduction caused hypo-sensitive to light irradiations. The loss-of-function mutant of this TF did not have any informative phenotype, probably due to presence of redundant factors. Presently, we are producing multiple knockdown mutants of this gene and its homologs in Arabidopsis.

P277 Role of dioxygenases in the phytoremediation of polycyclic aromatic hydrocarbons (PAHs) using the plant *Arabidopsis thaliana* as a model system

Juan Carlos Hernandez-Vega, Elizabeth Smith, Dawit Bezabih, Adán Colón-Carmona

Department of Biology, University of Massachusetts Boston

Anthropogenic pollution has a negative impact not only in human health and quality of life but also in fauna and flora of the ecosystems. Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 toxic compounds with two or more benzene rings, which are very stable, persistent in the environment and resistant to degradation. These compounds have been described as cytotoxic, teratogenic and/or carcinogenic. In Pseudomonas, PAHs can be modified by dioxygenases (DOXs), enzymes with the ability to introduce oxygen atoms that increase the reactivity of these compounds. The objective of this research is to investigate the involvement of plant DOXs in the chemical changes of PAHs once internalized by plants. Bioinformatics tools identified fifty-two of these enzymes on the TAIR database. Six *Arabidopsis thaliana* DOXs cDNAs were cloned and expressed in *E. coli* BL-21 cells. Normally E. coli cannot degrade PAHs as its sole carbon source unless these bacteria have been transformed with genes encoding enzymes capable of fulfilling that function. These clones were assayed for their ability to chemically modify indole to indigo, an indole biodegradation assay broadly used to identify bacteria capable of degrading PAH contaminants.

Preliminary results on Arabidopsis biodegrading DOXs will be presented. The direction of future work will be discussed.

P278 Cell expansion drives ethylene-induced differential petiole growth in *Arabidopsis thaliana*

Joanna Polko1, Martijn van Zanten1, Laurentius A C J Voesenek1, Anton J M Peeters1, Ronald Pierik2

1Plant Ecophysiology, Institute of Environmental Biology, Utrecht University. Sorbonnelaan 16, 3584 CA Utrecht, the Netherlands, 2Plant Ecophysiology, Institute of Environmental Biology, Utrecht University. Sorbonnelaan 16, 3584 CA Utrecht, the Netherlands.

Upward petiole movement, called hyponasty, is a very rapid reaction of plants in response to various external stimuli. Hyponastic growth brings rosette leaves to a more vertical position as a consequence of differential growth within a petiole. Many plant species use this strategy as a part of escape mechanism from unfavorable conditions such as submergence or proximity of neighbors.

The volatile hormone ethylene is one of the factors inducing hyponastic growth in *Arabidopsis thaliana*. The observed repositioning of leaves is a result of unequal growth rates between adaxial and abaxial sides of a petiole. Since the response is very rapid, it was suggested that cell elongation at the abaxial side of petiole is a driving force in this process. This hypothesis was supported by the microscopic analysis of epidermal imprints of Col-0 petioles subjected to ethylene exposure. Moreover, to complete this study, we used a T-DNA activation line (named DDD1) isolated in a genetic screen, which does not exhibit ethylene-induced hyponasty. Indeed, the cell zone responsible for differential growth in the wild type lacks elongation response in the case of DDD1. This knowledge gives us the opportunity to have a closer look at that particular population of cells in order to get more insights into the exact physiological and molecular mechanism underlying hyponastic growth in Arabidopsis.

P279 Nitrate transporter and pathogen resistance

Julie Dechorgnat1, Mathilde Fagard2, Anne Krapp1, Françoise Daniel-Vedele1

1INRA, IJPB, Unité de Nutrition Azotée des Plantes, F-78000 Versailles, France, 2Laboratoire des Interactions Plantes Pathogènes UMR 217 INRA/AgroParisTech/UPMC 75005 Paris, France

Nitrate, an essential element for plant growth and development, is taken up from soil solution by active transport through the plasma membrane of root cells. It is then translocated in the whole plant by different channels and transporters. To cope with large variations in nitrate concentrations in soils, two uptake systems coexist within plants, a low-affinity nitrate transport system (LATS) and a high affinity transport system (HATS). The *NRT1* and *NRT2* gene families are thought to be involved more specifically in either the LATS or the HATS system, although the *NRT1.1* gene was shown recently to be dual affinity nitrate transporter. In Arabidopsis, the *NRT2* family contains 7 genes, distributed on three chromosomes, and differentially regulated at the level of organ specificity or in response to environmental conditions.

This work is focused on one member of this family, *NRT2.6*. This gene is weakly expressed in all organs especially in the collar of young plantlets and in the tapetum of anthers.

The phenotypical analyses of a transposable element insertion mutant show that the loss of function affects neither the nitrate contents of plantlets nor the root uptake of nitrate. The mutant has viable pollen and normal segregation to descendants.

Public data showed that *NRT2.6* was induced by pathogen treatments. Therefore, we tested the mutant in response to *Erwinia amylovora* infection. This treatment induced rapidly the accumulation of *NRT2.6* transcripts in the same way as typical early genes like *NHO1*. The leaves of *nrt2.6* mutant inoculated with this bacterial pathogen show a higher susceptibility than wild-type linked to a better growth of bacteria in the mutant. This response is coupled with a reduced production of Reactive Oxygen Species without changing the expression rate of pathogen response marker genes.

We are currently investigating the role of *NRT2.6* in biotic stress response pathways.

P280 UBP14 is involved in root hair development under phosphate starvation in Arabidopsis

Margarete Müller1,2, Thomas Buckhout2, Thomas Altmann1

1IPK Gatersleben, 2Humboldt University Berlin

Under phosphate (Pi) starvation, plants increase the absorptive surface area of the root by increasing the number and length of root hairs. To identify new genes that are involved in this process, we screened an EMS-mutagenized Arabidopsis population for individuals that have no root hairs under Pi deficiency but develop normal root hairs under sufficient Pi supply. One of the derived mutant lines was characterized in more detail. The mutant developed only small bulges instead of root hairs under Pi starvation. Cryo-SEM images showed that the rhizodermis of the Pi-deficient mutant was deformed and that the root hair bulges had material accumulations at their tips. In the Pi-sufficient mutant, the shape of the root hairs was regular like in the wildtype. The number and position of root hairs was not changed in the mutant, neither in the presence nor in the absence of Pi, indicating that the Pi deficiency-induced root hair elongation is impaired rather than epidermal cell specification. Also other Pi starvation responses were altered in the mutant. The number of lateral roots was increased under Pi-sufficient and -deficient conditions. Furthermore, the anthocyanin content in the leaves of the mutant was increased under Pi deficiency. Backcross experiments showed a co-segregation of the mutant root hair phenotype with the increased lateral root number and anthocyanin content indicating that the alterations were caused by the same mutation. Map-based cloning of the mutation revealed a nucleotide exchange from C to T in the deubiquitinase gene *UBP14*, which causes a synonymous substitution. By screening a highly EMS-mutagenized population for further SNPs in the *UBP14* gene with TILLING, we identified two additional mutant alleles causing an impaired root hair elongation under Pi starvation.

P281 Identification of hyperactive forms of Arabidopsis MAP Kinases for the study of their target genes

Souha Berriri, Heribert Hirt, Jean Colcombet

URGVPlant Genomics Research, Evry, France

The involvement of phosphorylation in stress signalling has been clearly established.

Among the protein kinases and phosphatases involved in these processes, different members of the Mitogen-Activated Protein Kinases (MAPKs) play a prominent role. However, despite the abundance of available data, the exact roles and direct targets of the 20 MAPKs that exist in Arabidopsis are still not completely defined. In fact, in response to extracellular signals, more than one MAPK is typically concomitantly activated making it difficult to reveal their individual functions and downstream targets. Although many aspects of the mechanism of activation of MAP Kinases have been revealed, it is not known how to bypass or to emulate this mechanism. MAPK activation is obtained through an unusual mechanism that is unique to MAPKs (dual phosphorylation on neighbouring Thr and Tyr residues residing in a unique domain of the MAPK proteins called the phosphorylation lip). Ways to bypass or to mimic this activation mechanism were developed, but were only partially successful. Thus, the unique mode of activation hinders the production of intrinsically active variants. The first part of our work is to identify a constitutively active form of the well studied Arabidopsis MAPK, MPK6.

The second part of our work will be to produce transgenic lines that carry the constitutively active kinase under wild-type or inducible promoters. Apart from studying the phenotypic consequences of expressing a constitutively active version of a particular MAPK *in planta*, the molecular targets of the kinases shall be identified by microarray-based transcriptome analysis. Hereby, short time course experiments will be carried out upon induction of the constitutively active MAPKs.

Overall, the proposed work should provide direct information on all MAPK targets and should be an important contribution to the overall understanding of signal transduction in a complex system of a high eukaryotic model system such as *Arabidopsis thaliana*.

P282 Control of nitrogen remobilisation during leaf senescence and plant reproduction

Jeremy Lothier, Céline Diaz, Thomas Lemaitre, Laure Gaufichon, Anne Guiboileau, Céline Masclaux-Daubresse

IJPB-INRA centre de versailles, France

Leaf senescence is a key step for plant nutrient economy and nitrogen management. Leaf senescence is characterized by drastic metabolic changes. The major aim of such modifications is recycling and remobilisation of nutrients from the old and inefficient parts of the plant to the growing organs. Nitrogen is one of the major compounds mobilised from leaves to the rest of the plant during leaf senescence. To investigate physiology of nitrogen remobilisation during leaf senescence, we used Arabidopsis recombinant inbred lines (1; 3; 4) and accessions (2) showing different levels of leaf senescence severity. We also analysed mutants potentially affected in N-recycling such as (i) GS1;2 mutant in one of the cytosolic glutamine synthetases known to be induced during senescence and described as a potential actor of nitrogen remobilisation, and (ii) autophagy mutants implicated in cytoplasm and organelles recycling within lytic vacuole. Plants were grown in low nitrogen and short days, condition known to stimulate N-remobilisation and leaf senescence onset. We investigated N-remobilisation from leaf to leaf during sequential senescence and from rosette to seeds during monocarpic senescence using ^{15}N isotope tracing.

P283 Functional analysis of a plant specific RNA binding protein PSRP1 in Arabidopsis

Zhe Wu1,2, Danling Zhu1,2, Qian Yang1, Takashi Aoyama2, Tomohiko Tsuge2, Li-jia Qu1, Hongya Gu1

1National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing 100871, China, 2Institute for Chemical Research, Kyoto University, Gokasho Uji, Kyoto 611-0011, Japan

RNA binding proteins are generally considered to regulate gene expression at post-transcriptional level. The Arabidopsis genome encodes more than 200 putative RNA binding proteins, among which only a few has been functionally characterized. We are interested in a small group of plant specific RNA binding proteins (PSRPs), which are conserved among different plant species. In this study we conducted a functional analysis of the Arabidopsis gene *PSRP1*. The transcript of *PSRP1* was detected in all tested organs by using quantitative-PCR. Promoter-GUS analysis showed that the promoter activity of *PSRP1* could be mainly detected in developing trichome, root tip, lateral root cap, stomata, vascular tissues, and inflorescence. Over-expression of *PSRP1* resulted in an early flowering phenotype in the transgenic plants. Further analysis revealed that the expression of *FLC (FLOWER LOCUS C),* one of the major floral repressor, was significantly reduced in these early flowering plants. Interestingly, by yeast two-hybrid screening we identified an FPA-like protein that could interact with *PSRP1*, suggesting a novel role of *PSRP1* in flowering promotion.

We further found that the transcription of *PSRP1* was induced by 4ºC treatment, and that those plants over-expressing *PSRP1* exhibited enhanced tolerance to freezing. These data suggest that *PSRP1* is possibly involved in the regulation of different plant-specific processes.

P284 TINY GUY (TG), a DREB subfamily transcription factor, is potentially involved in stress response in Arabidopsis

Danling Zhu1,2, Zhe Wu1,2, Jigang Li1, Jia Wei1, Haiyang Wang 3, Takashi Aoyama2, Tomohiko Tsuge2, Hongya Gu1, Li-jia Qu1

1National Laboratory of Protein Engineering and Plant Genetic Engineering, College of Life Sciences, Peking University, Beijing 100871, China, 2Institute for Chemical Research, Kyoto University, Gokasho Uji, Kyoto 611-0011, Japan, 3Boyce Thompson Institute for plant research, Cornell University, Ithaca NY 14853 USA

The DREB transcription factors play vital roles in abiotic stress responses, such as cold, drought, and high salinity, through its regulation on growth and development in different plant species. In this study, we characterized a putative DREB transcription factor, *TINY GUY (TG)*, the transcript of which was highly accumulated in the late phase of seed maturation in Arabidopsis. *TG* was localized both in nucleus and in plasma membrane and functioned as a transcriptional activator in the yeast system. Further analysis showed that TG protein could directly bind to both the GCC and the DRE elements. Although the loss of *TG* function mutant did not display obvious phenotypes, constitutive over-expression of *TG* resulted in pleiotropic phenotypes, including defects in cotyledon and rosette leaf expansion, delay in flowering time, and sterility. Microarray analysis revealed 330 genes up-regulated by two-fold in the *TG* over-expression transgenic plants, approximately 200 of which contained the GCC and the DRE elements in the 1,000 bp upstream region of the promoters. These genes include drought and cold regulated genes, such as *COR47*, *COR15a*, *RD29a*.

Taken together, our data suggest that TG is possibly involved in regulating the expression of genes in response to environmental stresses.

P285 Phenotypical, molecular and biochemical characterization of new members from a TTL family involved in osmotic stress responses and ABA sensitivity

Naoufal Lakhssassi1, Veronica Gonzalez-Doblas1, Alicia Esteban1, Julio Omar Borsani2, Miguel Angel Botella Mesa1

1Biochemistry & Molecular Biotecnology Laboratory, University of Málaga, Spain, 2Agronomic University of Montevideo, Uruguay

Drought and salinity are the two most substantial adverse environmental factors encountered by land plants. Water deficit caused by drought and high salinity has been a major selective force in plant evolution as well as a constraint to crop productivity, limiting food production (Zhu, 2002). To cope with these environmental stresses, plants respond by initiating a number of physiological and molecular adaptive processes where abscisic acid (ABA) is a key regulatory determinant (Botella *et al*, 2005).

In a previous screening of Arabidopsis mutants affected in abiotic stress responses, we identified a Tetratricopeptide-repeat Thioredoxin-like 1 mutant (*ttl1*) that presented reduced tolerance to NaCl and osmotic stress which was apparent by a reduced root elongation, disorganization of the root meristem, and impaired osmotic responses during germination and seedling development (Abel *et al*, 2006). *TTL1* is involved in ABA-regulated responses, and regulates the transcript levels of several dehydration-responsive genes.

The *TTL1* gene encodes a novel plant protein with tetratricopeptide repeats and a region with homology to thioredoxin proteins. Based on homology searches to this *TTL1*, we have identified four *TTL* members in Arabidopsis genome with similar intron-exon structure and conserved amino acid domains in the encoded protein.

In this work, we generate double, triple and quadruple *TTL* mutants, and we show preliminary data related to their phenotypical, biochemical and molecular analysis.

1 Rosado, A. *et al* (2006). Plant Physiology, Vol. 142, pp 1113-1126.
2 Botella, M. A. *et al* (2005). Blackwell Publishing, Oxford, pp 38-62.
3 Zhu JK (2002). Annual Rev Plant Physiol Plant Mol Biol 53: 247-273.

P286 Circadian-regulation of wound responses as a potential mechanism to increase plant fitness

Michael Covington
Rice University, Houston, TX, USA

Plants, sessile organisms intimately tied to their environment, have evolved many ways to deal with changing local conditions. One coping mechanism is the circadian oscillator, producing self-sustained rhythms with ~24hr period. The importance of these rhythms has been demonstrated in both phytoplankton and higher plants: organisms with an internal clock period matched to the external environment possess a competitive advantage over those without. Other than enhanced photosynthesis, the mechanisms by which the circadian clock increases fitness are unclear. A mechanistic understanding could ultimately enable plant biologists to increase plant fitness, yield, and hardiness, as well as expand geographic regions of growth.

Many acute abiotic stresses are the direct result of daily light/dark cycles. As such, genes involved in the perception, signaling and/or responses related to such stresses might be expected to be under clock control. Insects and plant pathogens possess circadian systems that regulate daily activity and development, suggesting that biotic stresses are also likely to occur at predictable times of day.

Clock-controlled abiotic and biotic stress perception, signaling, and/or responses are, therefore, strong candidates for a mechanism by which circadian clocks increase fitness. Indeed, we have recently identified many groups of genes, responding to a variety of stresses that are significantly enriched for circadian-regulated genes. For example, we have demonstrated that transcript abundance for 56% of wound-induced genes and 100% of wound-repressed genes are circadian-regulated in unwounded plants (compared to 30% for all expressed genes in Arabidopsis). Furthermore, these two classes of genes are expressed at opposite times of day. We are in the process of (1) characterizing these circadian and wound-responsive expression patterns, (2) identifying mechanistic links between the clock and the wounding response, and (3) evaluating the physiological relevance of circadian wound responses.

P287 Biochemical characterization of two wheat phosphoethanolamine N-methyltransferase isoforms with different sensitivities to inhibition by phosphatidic acid

Ricarda Jost1,2, Oliver Berkowitz2,3, John Shaw2, Josette Masle2
1The University of Western Australia, Perth, WA, Australia, 2The Australian National University, Canberra, ACT, Australia, 3Murdoch University, Perth, WA, Australia

In plants, the triple methylation of phosphoethanolamine to phosphocholine catalysed by phosphoethanolamine N-methyltransferase (PEAMT) is considered a rate limiting step in the *de novo* synthesis of phosphatidylcholine (PC). Besides being a major membrane phospholipid, PC can be hydrolysed into choline and phosphatidic acid, (PA). While PA is involved in stress signalling, choline can be oxidised to yield the putative osmoprotectant glycine betaine. Loss of function mutants of one Arabidopsis PEAMT isoform, NMT1, show altered root development and alterations of programmed cell death.

Here we describe the cloning and biochemical characterization of a second PEAMT isoform in wheat that has a four times higher specific activity than the previously described WPEAMT/TaPEAMT1 and is less sensitive towards inhibition by S-adenosyl homocysteine, but more sensitive towards inhibition by phosphocholine. Both enzymes follow a sequential random Bi Bi mechanism and show mixed-type product inhibition patterns with partial inhibition for TaPEAMT1 and a strong noncompetitive component for TaPEAMT2. TaPEAMT2 mRNA is induced after prolonged cold treatment in both shoots and roots of young seedlings, with a bigger increase detected on total protein and activity levels. For the first time we demonstrate a direct repression of *in vitro* enzymatic activities by PA for both enzymes, with TaPEAMT1 being more sensitive than TaPEAMT2 in the physiological PA concentration range. Other lipid ligands identified in protein-lipid overlays are phosphoinositide mono- as well as some -diphosphates and cardiolipin. These results provide new insights into the complex regulatory circuits of phospholipid biosynthesis in plants and underline the importance of head group biosynthesis in adaptive stress responses.

This work has been funded by the Australian National University and the Grains Research and Development Corporation.

P288 Cell-type specific transcriptional stress responses in Arabidopsis roots

Anjali Iyer-Pascuzzi, Terry Jackson, Philip N Benfey
Department of Biology and IGSP Center for Systems Biology Duke University, Durham NC, USA

Plant survival is dependent on the ability to alter organ growth almost immediately upon response to abiotic stress. These developmental changes are the result of complex regulatory networks within specific cell types that compose whole organs. Understanding how stresses are perceived and translated into developmental changes is an important goal in plant biology, as it will facilitate both an understanding of cell identity and the identification of novel alleles for crop improvement. The Arabidopsis root's simple radial structure, well-defined cell types, and developmental plasticity make it a tractable system to study environmental stresses. Here we present an in-depth analysis of the root transcriptional response to 4 environmental stresses at the whole root, cell-type, and developmental stage levels. We show that nearly 12000 genes are stress-regulated in 5 cell types and 4 environmental conditions. We find that approximately 25% of cell-type specific genes are stress-specific. We find little evidence for a universal stress response and show that less than 1% of genes are differentially regulated by all 4 stresses. We show that cell-type specific transcriptional programs are environment dependent, as cell types have unique

stress-specific transcriptional profiles. Though cell types respond uniquely to different stresses, genes differentially regulated in one cell type under one condition are often stress-regulated in a different cell type by an alternative stress. Additionally, we find that sets of cell-type specific genes contain cell identity regulators necessary for that cell type's development. Using these sets we also predict new functions for root cell types and identify novel potential cell-identity regulators. Finally, we find known transcriptional networks operating in specific cell types and predict novel components of these networks.

P289 FIONA1 controls *CONSTANS (CO)* and *FLOWERING LOCUS C (FLC)* in summer annual

Miji Yeom1, Jeongsik Kim2, Yumi Kim1, Hyunmin Kim1, Hong Gil Nam1,3

1Division of Molecular Life Sciences and National Core Research Center for Systems Bio-Dynamics, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea., 2Department of Plant Cellular and Molecular Biology/Plant Biotechnology Center, Ohio State University, Columbus, Ohio 43210, USA, 3The I-BIO graduate program, POSTECH, Pohang, Kyungbuk 790-784, Republic of Korea.

Flowering is an important event from vegetative to reproductive stage. Recently, we reported that *FIONA1 (FIO1)* is essential for regulating period length in the Arabidopsis circadian clock. Also, *FIO1* affects the photoperiodic flowering pathway through increasing the expression of *CO*.

To get a better insight into the functional roles of FIO1 in regulation of flowering time, we performed microarray analysis using wild-type (Col) and *fio1-1* plant. The results showed that the expression of regulators in photoperiodic flowering such as *CO* is increased and expression of *FLC* is extremely decreased in *fio1-1* mutant. *FLC* is a key flowering regulator in the winter annual. However, our results showed that effects on *FLC* repression during the vernalization were minor in *fio1-1* mutant with an active *FRI* allele. These results indicate that FIO1 regulates the expression of *CO* and *FLC* in summer annual. Furthermore, we need to investigate how FIO1 controls the expression of *CO* and *FLC* in summer annual and to find the biological meaning of these results.

P290 Phosphoregulation of calcium dependent protein kinases (CDPKs) and changes in protein levels during ABA signaling mapped by Mass-Spectrometry

Maik Böhmer, Onur Erbilgin, Julian Schroeder

University of California San Diego, La Jolla, CA, US

The phytohormone abscisic acid (ABA) down regulates cell proliferation, causes cell cycle arrest, and regulates adaptation to abiotic environmental stresses such as drought, salinity and cold. In guard cells ABA mediates calcium-regulated and calcium-independent signaling and downstream ion channel activation and stomatal closure. Two CDPKs, CPK3 and CPK6, have been shown to regulate anion channel activity and stomatal closure in an ABA and calcium dependent manner (Mori, Murata *et al*, PLoS Biology, 2006). Experimentally imposing a calcium elevation in the cytosol of patch clamped guard cells without prior ABA incubation, however, did not lead to anion channel activation (Allen *et al*, 2002, Plant Cell; Siegel *et al*, Plant Journal, in press). ABA exposure enhances the ability of cytosolic calcium to activate S-type anion channels, which correlates with a calcium sensing priming hypothesis (Siegel *et al*, Plant Journal, in press; Young *et al*, PNAS, 2006). *In vivo* experiments followed by mass spectrometry suggest now that at least one CDPK is phosphorylated in a stimulus dependent manner.

In order to better understand the molecular basis of ABA signaling in a single cell system, we performed microarray analysis of an ABA time course and used a parallel technology at the protein level by applying a peptide labeling technique (iTRAQ) to an ABA responsive cell culture. We combined the information from microarrays and quantitative proteomics to identify proteins regulated by ABA and to determine the nature of their regulation. In initial experiments we identified and quantified more than 600 proteins. Among the proteins regulated by ABA were known ABA marker genes, such as *EM6* and *COR47*, but in some cases changes on the protein level were not accompanied by transcriptional changes or there was a reciprocal relationship, as for malate dehydrogenase, showing the value of a proteomic approach to complement microarray data sets.

P291 Light and temperature inputs into the circadian clock

Julia Foreman, Kelly L Stewart, Karen J Halliday

University of Edinburgh, UK

The circadian clock plays an important role in plants, enhancing performance and increasing vegetative yield. Light and temperature are both important signals allowing the entrainment of the circadian clock in Arabidopsis, either cue being sufficient for entrainment. The components of the central clock have been intensively studied, but most of this data is based on seedlings grown at 22°C. Recent data has shown that the role of central clock components can alter with temperature (Gould *et al*, 2006). Mutants in light receptors have been shown to alter the circadian clock. Phytochrome B is the major photoreceptor that operates in red light and lesions in this gene lead to a clock with a long period. Phytochrome B has also been shown to have a role in buffering the effect of temperature throughout the lifecycle of Arabidopsis.

We are interested in the interactions between light and temperature on the entrainment and architecture of the circadian clock. Data will be presented showing how the transcription of clock components alter with perturbations in temperature and light transduced via phytochrome B.

Gould PD, Locke JC, Larue C, Southern MM, Davis SJ, Hanano S, Moyle R, Milich R, Putterill J, Millar AJ, Hall A. Plant Cell. 2006 May;18(5):1177-87

P292 The activity of the bHLH transcription factor HFR1 is dependent on light and temperature

Henrik Johansson, Julia Foreman, Karen J Halliday
University of Edinburgh, UK

Plant growth and development are highly dependent on the ambient temperature.

Arabidopsis plants grown in warmer temperatures show an increase in hypocotyl elongation, leaf hyponasty and flower earlier than plants grown in cooler conditions.

Based on the phenotypic similarities of this response to the shade avoidance response (SAR) the bHLH transcription factor PIF4, which acts in the SAR, was recently identified as a major regulator of temperature responses.[1] Here we investigate the role of HFR1, another bHLH transcription factor also shown to play a role in the SAR,[2] in the response to changes in ambient temperature.

Our results show that depending on the light condition, HFR1 acts in one temperature stable and one temperature dependent pathway. We investigate the genetic relationship between *HFR1* and *PIF4* in monochromatic lights at different temperatures as well as their interactions with other components of the pathways.

1 Koini MA, Alvey L, Allen T, Tilley CA, Harberd NP, Whitelam GC, Franklin KA. Curr Biol. 2009 Mar 10;19(5):408-13
2 Sessa G, Carabelli M, Sassi M, Ciolfi A, Possenti M, Mittempergher F, Becker J, Morelli G, Ruberti I. Genes Dev. 2005 Dec 1;19(23):2811-5

P293 Photosynthesis light response curves in Arabidopsis plants under different water availability

João Lucas Coito1, Jorge Faria1, Jorge Marques da Silva1, Ana Rita Matos1, Adalcina Casimiro2, Anabela Bernardes da Silva1, Maria Celeste Arrabaça1, João Daniel Arrabaça1
1CEB/ Plant Molecular Biology and Biotechnology Lab, BioFig, Faculdade de Ciências, Universidade de Lisboa, Portugal, 2Departamento de Biologia Vegetal, Faculdade de Ciências, Universidade de Lisboa, Portugal

Photosynthesis light response curves were studied in four week-old Arabidopsis plants under progressive water deficit (WD) imposed by withholding water for up to two weeks using well-watered (WW) plants as controls in order to determine stress-induced susceptibility to photoinhibition. Gas exchange measurements showed that net photosynthetic rate (A) decreased sharply with the decrease in leaf relative water content (RWC). However, despite the lower A determined in leaves of WD plants a remarkable variation was also observed in WW plants. Similar results in *Medicago truncatula* plants were explained by a significant variation of stomatal conductance (gs) in WW plants with similar RWC (Nunes *et al*, in press). In those plants either the RWC or gs were different in WW and WD plants. In contrast, in Arabidopsis, WW and moderate WD had similar RWC, gs and A and differences were found only at severe WD conditions (RWC <60% and gs <0.1 mol m^{-2} s^{-1}). Photosynthesis light curves confirmed this pattern of A variation. Amax, photosynthetic apparent quantum yield (ϕ) and apparent light compensation point (LCP) changed significantly with RWC and gs but the effect of dehydration was only consistently obvious under severe WD. Besides the fact that all the photosynthetic parameters have changed, detailed analysis of each one suggested an increase in photorespiration in WD plants. The maintenance of similar RWC and gs in plants under a range of WD conditions and controls and the identical variation in A, Amax, ϕ and LCP highlight the Arabidopsis capacity to modulate photosynthesis to progressive WD conditions. This remarkable drought resistance is likely related to the early responses at the gene expression level.

Nunes *et al* Photosynthesis light curves: a method for screening water deficit resistance in the model legume Medicago truncatula. Annals Applied Biology (in press)

P294 Perturbing the Arabidopsis circadian clock system by limiting its light inputs

Bénédicte Wenden, László Kozma-Bognár, Qian Xing, Andrew Millar
Institute of Molecular Plant Science, Edinburgh, UK

In order to cope with the daily and seasonal changes in their environmental conditions, plants have a circadian clock, an endogenous mechanism that generates rhythms with an approximately 24-h period. These rhythms are generated by molecular oscillators that in Arabidopsis have been shown to consist of interlocking feedback loops involving many elements. In particular, light signals are essential to the synchronization of metabolism with the daily light/dark cycles, including the entrainment of the circadian clock.

At least eight photoreceptors were shown to be involved in the input pathways that entrain the circadian oscillator. Phytochrome A, in particular, is expected to be the only photoreceptor capable of perceiving the difference between darkness and low Red:Far Red (R:FR) ratio, hence it is the only active photoreceptor that can mediate FR light input to the circadian clock. Therefore, testing the circadian clock under strict FR light conditions provides an experimental framework to study the linkage between a single photoreceptor and its target clock function(s).

We analyzed gene expression for clock components under FR light using promoter::LUC (luciferase) reporters and quantitative real-time PCR. Our results uncovered the clock system's response to FR/dark conditions. The unexpected dynamics revealed by these experiments illustrate the power of highly defined environmental conditions, and the close interactions among its input photoreceptors.

P295 Metabolomics of ecotypic response to environmental variation

Chris Stewart
APS, University of Sheffield, Sheffield, UK

Atmospheric CO_2 concentration is currently higher than at any other time in the past 26 million years. It is

predicted to double within the next 100 years with far reaching implications to life on earth. How plants respond to this change and the mechanisms of this action is a hot topic of discussion. This research uses a metabolomic approach to investigate the ecotypic response to temperature and carbon dioxide partial pressure treatments of *Arabidopsis thaliana*.

P296 Toward the reconstruction of transcriptional regulatory pathways associated with hypoxia in Arabidopsis

Fu-Chiun Hsu1,2, Hsin-Ru Hung1, Hsiao-Ping Peng1,2, Ming-Che Shih1,2

1 Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan, 115 2 Department of Biological Sciences, University of Iowa, Iowa City, Iowa, USA 52242

Hypoxia response triggers multiple regulatory pathways in plants. AtMYB2 is a key transcription factor (TF) in the regulation of alcohol dehydrogenase (*ADH*) gene by oxygen deficiency. AtMYC2 is a hypoxia inducible TF that is involved in several abiotic stresses and abscisic acid (ABA) signalling pathways. Here, we show that AtMYB2 and AtMYC2 could cooperatively regulate *ADH* expression under hypoxia. We propose to profile AtMYB2 mediated hypoxia regulatory networks in a genome-wide scale. Genome-wide profiling of TFs to their targets has been successfully applied in simple eukaryotes, such as *Saccharomyces cerevisiae.* However, a major technical challenge in higher eukaryote is contributed by their higher genomic complexity. To establish a high-throughput platform, we have designed a strategy that is suitable for the reconstruction of transcription regulatory pathways in Arabidopsis. Clustering analysis of microarray data in hypoxia treated wild-type and *myb2-ko* Arabidopsis roots identified a list of genes that are coordinately regulated with ADH under hypoxia. Motif-finding algorithms identified conserved motifs in many of filtered genes, including MYB2 recognizing elements (MRE), and further screened putative MYB2 targets. Expressions of selected candidates were verified by real-time quantitative PCR in *myb2-ko* and *MYB2* over-expressing Arabidopsis. An electrophoretic mobility shift assay (EMSA) and chromatin-immunoprecipitation (ChIP)-PCR are established to confirm *in vivo* and *in vitro* interactions between MYB2 and its targets. Thus, the platform presented here reconstructs MYB2 mediated transcriptional pathways and provides extensibility to construct transcriptional networks among hypoxia inducible TFs.

P297 Isolation and characterization of Arabidopsis mutants with defects in acquired thermotolerance

Tsung-yuan Lee, Yee-yung Charng

Agricultural Biotechnology Research Center, Academia Sinica, Taipei, Taiwan

To identify new components involved in acquired thermotolerance (AT), we developed an assay to screen for Arabidopsis mutants that showed defective AT after long recovery following acclimation treatment (ATLR). From 38,638 EMS-mutagenized M2 seedlings derived from 4,800 M1 parents, 704 putative mutants with bleaching cotyledons but with true leaves evolved were selected to bulk up seeds for second round screening. Eventually, 4 mutants named *dht1-dht4*, (defective in heat tolerance) were confirmed. For further characterization, three additional heat shock (HS) regimes for measuring AT after short recovery (ATSR), basal thermotolerance (BT), and thermotolerance against moderate high temperature (TMHT), were also applied to these *dht* mutants in comparison with the phenotypes. Genetic analysis showed that *dht1/2* was caused by a single recessive mutation in *Hsp101* and could not be complemented by crossing to the *Hsp101* knockout mutant, which suggests that *Dht1/2* and *Hsp101* are the same allele. Hypocotyl elongation assay of *dht* mutants showed defect in *dht1/2* under ATLR condition but not ATSR and the phenotype of *dht1* may be caused by Hsp101 protein stability declined. Genetic analysis of F2 seedlings showed *dht3* is a dominant mutant and *dht4* is a recessive mutant. Using map-based cloning, we mapped the *dht4* mutation to *AtCHLG* (At3g51820), a chlorophyll synthase. A single G-to-A mutation, result in a Gly-to-Arg substitution at amino acid 217 of the *AtCHLG* gene. The results of *dht1/2* and *dht4* mutants indicate the feasibility of our forward genetic approach in identifying important and novel components for thermotolerance.

P298 FKBP proteins are important determinants of intracellular acid stress tolerance in yeast and in Arabidopsis

Gaetano Bissoli, Regina Niñoles, Consuelo Montesinos, Guillem Hueso, M Dolores Planes, Ramón Serrano, José M Mulet

Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia-C.S.I.C., Camino de Vera s/n, 46022 Valencia, Spain

Our previous work in yeast has demonstrated that overexpression of *FPR3*, an FKBP immunophilins, conferred tolerance to intracellular acid stress generated by permeable weak organic acids such as acetic and sorbic acids. FK506 binding proteins or FKBPs where originally identified as the cellular targets of the immunosupressant drugs Rapamycin and FK506. FKBPs are peptidyl-prolyl cis-trans isomerases (PPIase EC 5.1.2.8) that catalize the rapid isomerization of prolyl bonds from the *cis* to the *trans* configuration. FKBPs are ubiquitous proteins that can be found as a single catalytic domain (or single domain immunophyllins) proteins or being part of more complex proteins. To assess the implication of FKBP proteins in weak acid tolerance in Arabidopsis we have generated lines of *Arabidopsis thaliana* overexpressing two different FKBP proteins: yeast FPR1 or Arabidopsis FKBP65 (ROF2). In presence of acetic acid the transgenic lines grew better than wild type plants. On the other hand an AtFKBP65 loss-of-function mutant line showed a decreased growth compared to wild type in the same conditions. We have also screened for different phenotypes and found that AtFKBP65 over-expressing plants showed enhanced response to ABA.

P299 Isolation and characterization of an *Arabidopsis thaliana* mutant resistant to norespermidine

Rosa Porcel, Carolina Abril, Santiago Alejandro, Ramón Serrano

Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia-C.S.I.C., Camino de Vera s/n, 46022 Valencia, Spain

The homeostasis of cations is a fundamental activity of living cells, with both permissive and regulatory roles in many cellular functions. Transport of metal and alkali cations across plant plasma and organelle membranes is essential for plant growth, development, signal transduction and nutrient utilization, and also for extrusion and compartmentalization of toxic ions under stress conditions. One approach to identify novel regulators of cation transport consists of the identification of genes that upon gain of function improve tolerance to toxic cations. Gain-of-function mutants obviate genetic redundancy and can identify bottlenecks in biological pathways. Norespermidine is a non-metabolizable polyamine, which is toxic for plants. We have used this polyamine because it is toxic at low concentrations so we avoid the osmotic effect. Previous work in our lab has determined that the *QSO2* gene (*At1g15020*) isolated using norespermidine as a selection agent, encodes a member of the quiescin-sulfhydril oxidase family and it is an important determinant of potassium homeostasis (Alejandro *et al*, 2007). In the present work an activation-tagging mutant seed collection was screened and we identified a mutant line resistant to norespermidine named *par4* (polyamine resistant). *par4* mutant is also resistant to weak organic acids, hydrogen peroxide, cold and accelerated aging of seeds when compared to wild type plants. Phenotypical observation also found that the mutant exhibited a bigger size with less number of leaves and earlier bolting than wild type plants. Morphometrical analysis showed that the *par4* mutant presented bigger cotyledons area and perimeter. In addition, the length and number of roots were greater in *par4* mutant. T-DNA localization and genetic characterization will be necessary to elucidate the role of the mutated gene in cation homeostasis in plants.

Alejandro S, Rodríguez PL, Bellés JM, García-Sánchez, MJ, Fernández JA, Serrano R. (2007). An Arabidopsis quiescin-sulphydryl oxidase regulates cation homeostasis at the root symplast-xylem interface. EMBO J 26: 3203-15.

P300 Arabidopsis *wat1*-1D mutant reveals a role of a C2 domain containing protein in intracellular pH homeostasis

Regina Niñoles1, Santiago Alejandro, Christoph Plieth2, Ramón Serrano

1Instituto de Biología Molecular y Celular de Plantas, Universidad Politécnica de Valencia-CSIC, Camino de Vera s/n, 46022 Valencia, Spain, 2Zentrum für Biochemie und Molekularbiologie, Universität Kiel, Am Botanischen Garten 9, 24118 Kiel, Germany

The homeostasis of intracellular pH is a fundamental activity of living cells because this parameter modulates crucial systems including metabolism, membrane transport, vesicle transport and cell growth and death. In plants the plasma membrane H^+-ATPase, K^+ transport, phosphofructokinase, inorganic nitrogen assimilation and carboxylation-decarboxylation reactions have previously been identified as crucial factors in pH homeostasis. However, little is known about the signal transduction pathways involved in their regulation. Intracellular acidification ensues from many abiotic stresses such as K^+ starvation and exposure to acid rain. Therefore, understanding acid tolerance has both scientific and biotechnological relevance. In order to identify new components of the Arabidopsis pH regulation system, an activation-tagged mutant seed collection was screened using acetic acid as a selection agent. The dominant mutant *wat1-1D* (Weak Acid Tolerant) is much more resistant to intracellular acid stress generated by weak acids such as acetic, propionic and sorbic acids, and this tolerance correlates with its T-DNA insertion. *wat1-1D* mutant is sensitive to toxic cations such as norspermedine and lithium. The T-DNA insertion of this mutant was localized next to the At3g55470 gene (*WAT1*) causing its overexpression. Transgenic lines over-expressing *WAT1* gene showed improved seed germination under intracellular acid stress while knock-out mutants in this gene exhibit acid sensitivity. Measurements of intracellular pH with a pH-sensitive derivative of the green fluorescent protein (pHluorin) indicated that mutant root cells maintain a higher intracellular pH than wild type cells, both in the absence and in the presence of weak acids. *WAT1* encodes an orphan C2 domain, a domain usually included in complex proteins such as protein kinases and phospholipases and that binds calcium and acidic phospholipids. These results suggest a cross-talk between calcium signalling and pH homeostasis. The identification of the WAT1 interacting proteins will help to clarify the signalling pathway involved. Our working hypothesis is that WAT1, via an unknown interacting protein kinase, activates the plasma membrane H^+-ATPase in response to calcium signals.

P301 Local and systemic regulation of phosphate starvation responses in Arabidopsis

Margarete Müller1,2, Thomas Buckhout2, Bikram Datt Pant3, Wolf-Rüdiger Scheible3, Thomas Altmann1

1IPK Gatersleben, 2Humboldt University Berlin, 3MPIMP Golm

Under phosphate (Pi) starvation, plants induce morphological and physiological acclimation responses in the root that increase the explored soil volume, help to acquire Pi from insoluble components, and are involved in the remobilization of Pi within the plant. To find out if some of these responses are controlled by the Pi status of the shoot or by the local Pi availability, we conducted split root experiments with plants that had either a Pi-sufficient or deficient shoot. This approach allows all possible combinations of local and systemic sufficiency and deficiency signals. The activation pattern of the respective deficiency response in the split root parts defined six regulatory groups. Root growth inhibition was solely controlled by external Pi availability. The increase in the root hair number depended on the external Pi supply but could also be triggered by a systemic deficiency signal. The

phosphate starvation-inducible gene expression could be divided into two local and two systemic groups. The local groups responded mainly to the Pi availability in the soil and to the Pi pool within the root; they were often expressed in the vasculature and outer cell layers of the root. The systemic groups responded mainly to the shoot Pi status but also to the Pi pool within the root and were often active in the vasculature of the root and shoot. The local groups were enriched in catabolic enzymes and the riboregulators *At4* and *IPS*1, whereas, the systemic groups exclusively expressed the different miR399 species.

P302 Expression of alternative oxidase genes in Arabidopsis leaves under progressive drought stress

Ana Rita Matos1, Dave Pinxteren1, João Lucas Coito1, Anabela Bernardes da Silva1, Jorge Marques da Silva1, Maria Celeste Arrabaça1, Adalcina Casimiro2, João Daniel Arrabaça1

1CEB/Plant Molecular Biology and Biotechnology Lab, BioFIG, Faculty of Sciences, University of Lisbon, Portugal, 2Faculty of Sciences, University of Lisbon, Portugal

Water deficit is a major abiotic stress affecting plant growth and productivity. The effects of drought on photosynthesis have been extensively studied, whereas the impact of this stress on plant respiration is much less understood, despite the metabolic interdependence between chloroplasts and mitochondria. Besides the cytochrome c oxidase plants have an alternative oxidase (AOX), which accepts electrons directly from ubiquinol, reducing ATP yield and dissipating energy as heat. AOX is stress-inducible and may prevent the accumulation of reactive oxygen species. Several studies have used Arabidopsis to unravel the molecular and physiological mechanisms underlying plant responses to drought. However, in most cases, water stress was imposed by rapid desiccation of up-rooted plants or detached leaves. The aim of our work was to investigate the effects of progressive drought stress on the expression of the *AOX* genes in Arabidopsis leaves. Watering was withheld for up to two weeks in four week-old Col-0 plants. The leaf water status was followed by measuring the relative water content (RWC) and the soil water content was also recorded. A time-course analysis of gene expression was performed by RT-PCR. AOX is encoded in Arabidopsis by five genes. AOX1a has previously been shown to be the major AOX isoform in Arabidopsis leaves and also to respond to a variety of stresses. *AOX1a* transcripts are present in control leaves and the expression of this gene is up-regulated at the early stages of the treatment, when the RWC of the leaves is similar to that of control plants. The amount of *AOX1a* transcripts is higher in severely stressed leaves. *AOX1c, AOX1d* and *AOX2* are differentially regulated in response to water deficit whereas transcripts of *AOX1b* were not detected. Up-regulation of *AOX* genes may contribute to adjust carbon assimilation under drought stress.

Acknowledgements: ARM was financed by FCT (SFRH / BPD/ 26824 / 2006)

P303 The *early bird 1* reveals a new cog in the circadian clock

Mikael Johansson1, Harriet McWatters2, László Bakó1, Anthony Hall3, Andrew J Millar4, Maria E Eriksson1

1Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, SE-901 87 Umeå, Sweden, 2Department of Plant Sciences, Oxford University, Oxford OX1 3RB, England, 3School of Biological Sciences, Liverpool University, Liverpool L69 7ZB, Merseyside England, 4Institute of Molecular Plant Sciences, Edinburgh University, Edinburgh EH9 3JH, Midlothian Scotland

The daily transitions between light and dark that most organisms are exposed to on the planet Earth have led to the evolution of a system of molecular mechanisms that is called the biological, or circadian, clock. The circadian clock is an important coordinator of inner metabolic events with external conditions. In *Arabidopsis thaliana* the circadian clockwork is believed to comprise of at least three interconnected negative feedback loops. The central loop contains the single Myb-domain transcription factors *CIRCADIAN CLOCK ASSOCIATED 1 (CCA1)* and *LATE ELONGATED HYPOCOTYL (LHY)* which repress the positive element *TIMING OF CAB EXPRESSION 1 (TOC1).*

We have found that the novel clock mutant early bird1 shortens clock period of *CHLOROPHYLL A/B BINDING PROTEIN 2* fused to *LUCIFERASE (CAB2:LUC)* across all light intensities and wavelengths tested. The mutation does not affect hypocotyl elongation implying its effect is restricted to the clock. These defects remain following light and temperature entrainment, suggesting EBI1 acts near the central loop. Moreover, *cca1lhyebi1* triple mutant shows an extremely short period phenotype, with a sustained rhythm, indicating that EBI1 acts redundantly of CCA1 and LHY and that its role in the clock is related to pace rather than rhythmicity. A red light pulse experiment over 36 h showed an altered gating where the *ebi1* mutant responds to light 4 h before subjective dawn, suggesting that EBI1 function may be acting at this point. We have cloned and carried out biochemical studies on EBI1's interactions with characterized clock components, which will be discussed. In conclusion EBI1 is a novel clock component that acts to control the pace of the central clock loops.

Plant Defence

P304 Metabolite screening to identify *in vivo* ligands for glutathione transferases

David Dixon, Robert Edwards

Durham University, Durham, UK

Arabidopsis possesses a 55 member glutathione transferase (GST) superfamily, with the two largest clades (phi class, 13 members; tau class, 28 members) being functionally uncharacterised apart from a single member involved in flavonoid transport. However, many of these enzymes show strong induction on biotic and abiotic stress, can be extremely abundant enzymes, and in crops

are important for herbicide selectivity, so their endogenous roles are important. The large gene family size precludes most genetic studies, but biochemical studies have proved more fruitful. We have therefore cloned 51 GSTs expressed in Arabidopsis for further study. Each enzyme was expressed as an N-terminally *Strep*-tagged polypeptide in *E. coli,* where most were successfully purified and subsequently assayed for a range of activities. The metabolites of bacteria expressing each GST were profiled by HPLC-UV-MS and a diverse selection of unexpected compounds were identified as accumulating on GST expression, with subsequent studies showing this accumulation was due to tight binding to certain GSTs. These compounds included heterocycles and glutathionylated derivatives of porphyrins and fatty acids. The most interesting GSTs were then expressed using a custom vector as *Strep*-tagged polypeptides both transiently in *Nicotiana benthamiana* and stably in Arabidopsis. Enzymes were subsequently purified and examined for bound ligands by HPLC-MS, allowing a very diverse range of ligands to be identified. These ligands included hydrophobic flavonoids, glutathione-conjugated lignanamides, glutathione-conjugated oxygenated fatty acids and mixed di- and poly-sulfides between glutathione and 3-methylthioindole. Where possible, binding was confirmed and further characterised using *in vitro* binding assays. Based on these screens, potential functions for plant GSTs in primary and secondary metabolism, defence and signalling are discussed.

P305 TGA transcription factors negatively regulate PAMP signaling cascades

Katja Rindermann, Christiane Gatz

Georg-August-University Göttingen, Germany

Three redundant members of the TGA family of bZIP transcription factors play a crucial role in mediating the salicylic acid-dependent defense response “systemic acquired resistance”. Here we will present evidence that these factors are also involved in repressing the responses to pathogen associated molecular pattern (PAMPs). This pathway is hijacked by the Pseudomonas virulence factor coronatine, which activates a TGA-dependent pathway to suppress PAMP mediated processes like e.g. closure of stomatal openings.

P306 A member of the Arabidopsis polygalacturonase gene family modulates leaf development and responses to biotic and abiotic stress

Rana Poor Aiiouby1, Sven Friehe2, Elmon Schmelzer3, Alan Slusarenko2, Nikolaus L Schlaich2

1RWTH Aachen University, Aachen, Germany, 2RWTH Aachen University, Aachen, Germany, 3Max-Planck Institute for Plant Breeding Research, Cologne, Germany

Screening Arabidopsis plants transformed with a 35S::PCC1 overexpression construct, we serendipitously isolated a mutant that showed abnormally shaped leaves and was reduced in stature. The mutant was selected for detailed characterization and the phenotype was shown to be due to insertion of the T-DNA in the promoter of the At3g07970 gene, predicted to encode a polygalacturonase (PGase), rather than PCC1 overexpression because i) other characterized 35S::PCC1 transformants resembled the wildtype, ii) an independent T-DNA insertion line from the GABI-Kat collection in the promoter of the same gene recapitulated the mutant phenotype and iii) constitutive expression of the wildtype *PGase* gene in the GABI-Kat mutant restored the wildtype phenotype. Physiological characterization of the pgase mutants revealed that, while gene-for-gene incompatibility to *Hyaloperonospora arabidopsidis* (formerly *H. parasitica*) was unaffected, the mutant plants were hypersusceptible to a virulent race of *H. arabidopsidis* and to temperature stress. Our data reveal a critical role for this *PGase* in leaf development and resistance to both biotic and abiotic stressors.

P307 Subtle changes in the sequence of the *as-1-like* element have a strong impact on the regulation of the *PR-1* promoter

Sebastian Pape, Corinna Thurow, Christiane Gatz

1Albrecht-von-Haller Institute for Plant Science, Untere Karspuele 2, 37073 Göttingen, Germany

The Arabidopsis *PR-1* gene belongs to a subset of genes upregulated during “systemic acquired resistance” (SAR), a plant defense response against a broad spectrum of pathogens mediated by the signaling molecule salicylic acid. The expression of *PR-1* is strictly dependent on NPR1, SNI1 and TGA transcription factors. NPR1 is a key activator of SAR, which interacts with TGA transcription factors. When the repressor SNI1 is absent, the function of NPR1 is no longer essential for *PR-1* induction indicating that an NPR1/SNI1 independent induction pathway exists.

In this study it is demonstrated that the TGA factor recruiting *as-1*-like element is important for NPR1- and SNI1 dependent regulation. When both TGACG motifs are mutated the *PR-1* promoter still shows inducibility. This indicates that neither TGA factors nor NPR1 are required for induction of this promoter derivative. As this promoter is NPR1-independent, we hypothesize that the repressor SNI1 is recruited to the *as-1*-like element in the wildtype promoter context and mutation of the TGA recruitment sites abolishes NPR1- and SNI1 regulation at the same time. Further evidence for this hypothesis was obtained with a promoter construct carrying a shortened linker sequence simulating the conserved 12bp distance between the two TGA binding motifs of NPR1- and SNI1 independent *as-1* elements known from the 35S- or the GST6 promoters. Though both TGACG motifs are present only a faint induction occurs as SNI1 may constitutively interfere with activation. The mutated construct regains inducibility when transformed in *sni1-1* illustrating that SNI1 is indeed repressing the promoter. The repression cannot be released as NPR1 is no longer able to be recruited to the mutated *as-1* element. It is concluded that the distance between the two TGACG motifs determines whether NPR1 is recruited to the *as-1* element or not. In contrast substitution of the *as-1*-like element against the *35S as-1* element leads to a 400-fold increased

basal- and 40-fold increased induced expression levels indicating that this promoter does not recruit SNI1.

P308 The RNA silencing suppressor protein encoded by cucumber mosaic virus perturbs host jasmonate and salicylate responses

Mathew G Lewsey1, Alex M Murphy1, Daniel MacLean2, Neil Dalchau1,3, Mark Bennett4, Michael Moulin1, Alison G Smith1, Glen Powell4, David E Hanke1, John P Carr1

1Department of Plant Sciences, University of Cambridge, Cambridge, UK, 2Sainsbury Laboratory, Norwich Research Park, Norwich, UK, 3Department of Engineering, University of Cambridge, Cambridge, UK, 4Plant and Microbial Sciences, Imperial College London, South Kensington Campus, London, UK

The 2b counter-defence protein of the aphid-transmitted cucumber mosaic virus disrupts plant anti-viral mechanisms mediated by RNA silencing and salicylic acid (SA). To better understand the mechanisms of defence and counter-defence, we investigated SA-regulated gene expression in *2b*-transgenic Arabidopsis. The 2b protein caused an enhancement of the expression of SA-regulated genes, supporting a previous study. CMV infection induced accumulation of SA in host plants, but transgenic expression of *2b* protein reduced this accumulation. This suggests some habituation of SA-mediated defence mechanisms to the virus by transgenic expression of a viral protein. Extensive crosstalk exists between defence signalling mediated by SA and that mediated by jasmonates (jasmonic acid and its derivatives). Consequently, we also investigated jasmonate-regulated gene expression in *2b*-transgenic plants. Surprisingly, the *2b* protein disrupted expression of 90% of genes regulated by jasmonates. The accumulation of jasmonic acid in wounded *2b*-transgenic plants was also examined. This is the first demonstration that a virus-encoded silencing suppressor has such extensive effects on jasmonate-regulated signalling.

P309 Dissection of RPM1-mediated disease resistance using a novel genetic screen

Mindy Roberts1, Tim Eitas1, David Hubert2, Jeff Dangl1

1UNC Chapel Hill, North Carolina, USA, 2BASF, Research Triangle Park, North Carolina, USA

NB-LRR proteins, named for their nucleotide-binding and leucine-rich domains, and bacterial type III effector proteins are key components of plant-pathogen interactions. The model bacteria *Pseudomonas syringae* delivers these effector proteins into plant host cells via the type III secretion system. If the host plant expresses the corresponding disease resistance protein to perceive the presence of the effector, recognition occurs. This recognition triggers various defense responses, frequently culminating in the hypersensitive response, a type of programmed cell death. One relatively well-characterized example of such a resistance relationship is the response of the Arabidopsis NB-LRR protein RPM1 to the bacterial effector AvrRpm1. Multiple other proteins are known to be required for proper RPM1-mediated defense responses. These proteins are frequently involved in regulating the stability and turnover of the NB-LRRs (e.g. HSP90, below).

A novel genetic screen was performed to find Arabidopsis mutants that do not have a proper defense response when challenged with *P. syringae* carrying *avrRpm1*. Mutants isolated from this screen are called lras, for loss of recognition of *avrRpm1*. We are currently in the process of identifying the gene responsible for one mutant identified from this screen, *lra8*, via both map-based cloning and whole-genome sequencing. *lra8* is a single, recessive locus defined by two alleles with decreased resistance to *Pto*DC3000 *(avrRpm1)*, as measured by both bacterial growth and ion leakage, as well as reduced RPM1 protein accumulation. In addition to its role in RPM1 function, *lra8* also exhibits a partial loss of function for RPS5, an additional NB-LRR protein. After identification of the gene, I will use epistasis analysis, GFP localization, co-immunoprecipitation, and yeast 2-hybrid to position *LRA8* within the network of genes already known to be important for proper RPM1 function, such as *RAR1*, *SGT1b*, and *HSP90*.

P310 The role of protease inhibitors in the genetic network restricting pathogen-induced necrosis

Kristin Laluk1, Hongli Luo1, Fengming Song2, Tesfaye Mengiste1

1Purdue University, West Lafayette, IN, USA, 2Zhejiang University, Hangzhou, Zhejiang, China

Biotrophic plant pathogens require living cells to complete their lifecycle whereas necrotrophs kill host cells to obtain nutrients, often inducing expanding necrotic lesions. When hosts fail to restrict lesions, disease culminates in the decay of the entire plant. The genetic factors conferring resistance to necrosis caused by necrotrophic infection are poorly understood. *BOS1*, an R2R3MYB transcription factor, was identified as a crucial component of Arabidopsis defense against necrotrophic fungi. A network of BOS1 interacting proteins and genes with *BOS1*-dependent expression were identified through yeast two hybrid screens and expression profiling. *La vita BOS1 (LAB1)*, a strong BOS1 interactor, encodes a RING E3 ligase resembling mammalian inhibitor of apoptosis proteins (IAPs). LAB1 restricts necrosis caused by *B. cinerea* and α-picolinic acid (PA toxin), a known inducer of cell death in animal and plant cells. LAB1 interacts with Arabidopsis *CYSTATINA (CYSA)*, a cysteine protease inhibitor. This network presents a framework analogous to mammalian apoptosis regulation in which interactions of IAPs, cysteine proteases and cystatins control cell death. The Arabidopsis cysa loss of function mutant exhibits increased susceptibility to necrotrophic infection as well as sensitivity to abiotic stress and plant hormones typically associated with plant defense signaling. A second protease inhibitor gene *BASS1 (B. cinerea and A. brassicicola SUSCEPTIBLE SERINE PROTEASE INHIBITOR 1)* was identified based on its increased expression in the *bos1* mutant. *BASS1* is highly expressed in tissues undergoing necrosis, hypersensitive response and

developmental cell death (senescence). Loss of *BASS1* increased susceptibility to necrotrophic infection as well as delayed flowering. *BASS1* exhibits serine protease inhibitor activity with a weak affinity for cysteine proteases. Together, our data suggest that protease inhibitors play an important role in limiting cell death caused by environmental and developmental cues.

P311 Ecological genetics of microbial sensing in British populations of *Arabidopsis thaliana*

Eric Holub1, Robin Allaby1, Laura Rose2

1University of Warwick, UK, 2University of Munich, DE

Molecular genetics of *Arabidopsis thaliana* has revolutionized crop breeding over the past two decades of plant research. Molecular insight from this geographically widespread wildflower may also contribute significantly to important aspects of conservation biology, aimed at improving our ability to protect botanical biodiversity and precious habitats (Holub 2008. Eur J Plant Pathol 122:91-109; Holub 2007. Curr Opin Plant Biol 10:415-424). For instance, the genetic resilience of a given plant species is expected to depend on the adaptive ability of local populations to manage symbioses, in both minimizing the impact of disease caused by parasites and in reaping benefits from association with mutualistic symbionts. Several families of pathogen receptor-like proteins (typically containing a leucine rich repeat domain) have evolved to provide plants with the innate means of sensing the earliest stages of microbial infection, and then stimulating an appropriate biochemical response. Receptor proteins that stimulate defense against potential parasites are particularly well understood in the laboratory environment of *A. thaliana*. However, the importance of these proteins in the molecular ecology of a wild or domesticated plant species presents an emerging frontier for research. Genetic resources, high density DNA sequence information (e.g., Arabidopsis HapMap and 1001 Genome projects), and protected field sites are currently being assembled which enable exploration of spatial and temporal distribution of natural variation in these R proteins, and ultimately whether these proteins do indeed provide genetic resilience that is innately embedded in local populations of a host species.

P312 Regulation of a bean proline-rich protein gene expression during defense response in transgenic Arabidopsis

Songhita Das1, Mona Mehdy2

1Institute of Cell and Molecular Biology, University of Texas at Austin, USA, 2Institute of Cell and Molecular Biology, University of Texas at Austin, USA

During the defense response to pathogen attack & wounding, plant cells modify their walls to produce an effective barrier to pathogen invasion. This involves both up-and down-regulation of cell wall protein genes. One down-regulated gene is the French bean proline-rich protein, PvPRP1.The PvPRP1 protein is hypothesized to be less cross linked due to low tyrosine content compared to other tyrosine-rich proline-rich proteins, and to not contribute to cell wall strengthening. *PvPRP1* mRNA half life is reduced in elicitor treated cells. The 3'-UTR of *PvPRP1* has two AUUUA motifs. AUUUA motifs are often known to regulate mRNA half life. A 50-kD protein PRP-BP specifically binds to a 27bp region containing the first AUUUA motif in cellular extracts, and is hypothesized to contribute to *PvPRP1* mRNA down-regulation. To further study this mechanism, three different *PvPRP1* constructs were introduced in Arabidopsis: (1) the full-length with 3'-UTR (2) a truncated 3'-UTR containing only the first AUUUA motif (3) a truncated 3'-UTR without AUUUA motifs. Jasmonate serves as a signal molecule for gene induction and repression. Earlier work on an Arabidopsis homologue of *PvPRP1*, *AGP31*, showed 30% decrease in mRNA within 8h to methyl jasmonate treatment. Currently, the above *PvPRP1* transgenic lines are being treated with methyl jasmonate and are being analyzed by Q-PCR. The PRP-BP has also been cloned and transformed in Arabidopsis to study further mRNA regulation. My work will help in understanding mRNA destabilization and may lead to improved disease resistance of crops in future.

P313 The Arabidopsis CBP60g and h proteins define a critical node in salicylic acid signaling

Lin Wang, Kenichi Tsuda, Masanao Sato1, Fumiaki Katagiri, Jane Glazebrook1

University of Minnesota, St Paul, MN, USA

We have studied two closely-related members of the Arabidopsis calmodulin-binding protein 60 (CBP60) gene family, designated g and h. CBP60g is a bona fide calmodulin (CaM)-binding protein, but CBP60h lacks a CaM binding site and does not bind CaM. Plants with cbp60g mutations have a defect in MAMP-induced salicylic acid (SA) production at nine hours after challenge, and show enhanced disease susceptibility (eds) to *Pseudomonas syringae pv. maculicola* strain Psm ES4326. However, SA levels 24 hours after Psm ES4326 infection are normal. Site-directed mutagenesis experiments showed that CaM binding is required for the functions of CBP60g in SA production and limitation of Psm ES4326 growth. Plants with *cbp60h* mutations have a defect in SA production following Psm ES4326 infection, and are also eds to Psm ES4326. However, SA levels at nine hours after a MAMP challenge are normal. Double *cbp60g,h* mutations cause a severe defect in SA production during a MAMP response, infection by *P. syringae pv. tomato* strain Pst DC3000 *avrRpt2*, or infection by Psm ES4326. They are also severely eds at a level greater than the SA synthesis mutant *sid2*, and comparable to highly pleiotropic *pad4* mutants. Thus, the two genes define a critical and partially redundant function in SA signaling, with CBP60g playing a more important role during a MAMP response, and CBP60h playing a more important role later during pathogen infection. Expression profiling revealed that the *cbp60g,h* double mutation affects all the genes affected by *sid2*, as well as a subset of SID2-independent genes affected by *pad4*. This analysis places the *CBP60g,h* node downstream of the

PAD4/EDS1 node and upstream of SA synthesis in the plant defense signaling network.

P314 MKK1 and MKK2 synergistically regulate developmental and disease resistance

Peter Morris1, Jin-Long Qiu2, Lu Zhou1, Byung-Wook Yun3, Henrik Nielsen4, Berthe Fiil2, Klaus Petersen2, Gary Loake3, John Mundy2

1Heriot-Watt University, Edinburgh, UK, 2University of Copenhagen, Denmark, 3Edinburgh University, UK, 4Technical University of Denmark, Kgs. Lyngby, Denmark

The *Arabidopsis thaliana* MKK1 and MKK2 MAP kinase kinases have been implicated in biotic and abiotic stress responses as part of a signalling cascade including MEKK1 and MPK4. We show that the double loss-of-function mutant (*mkk1/2*) of *MKK1* and *MKK2* have marked phenotypes in development and disease resistance similar to those of the single *mekk1* and *mpk4* mutants. Since *mkk1* or *mkk2* single mutants appear wild type, basal levels of MPK4 activity are not impaired in them, and MKK1 and MKK2 are in part functionally redundant in unchallenged plants. We have confirmed and extended these findings by physiological, biochemical, genetic and molecular analyses implicating the MKK1 and MKK2 kinases in jasmonate- and salicylate-dependent defense responses, mediated in part via the MPK4 substrate MKS1. In addition, transcriptome analyses delineate both overlapping and specific effects of these kinases on global gene expression patterns, demonstrating both redundant and unique functions for MKK1 and MKK2.

P315 GABA regulates E-2-hexenal responses and Pseudomonas susceptibility

Rossana Mirabella1, Duck Hwan Park2, Han Rauwerda 3, Michel Haring1, Alan Collmer2, Robert Schuurink1

1Plant Physiology, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam, 2Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 4853 USA, 3Microarray Department, University of Amsterdam, Science Park 904, 1098 XH, Amsterdam

Plants respond to herbivore damage or pathogen infection by emitting a bouquet of C6-volatiles from vegetative tissues. Uninfested plants, exposed to these volatiles, respond with transcriptional and metabolic changes related to defense mechanisms, indicating that these volatiles act as signaling molecules regulating the plant defense responses against pests. However, to date the mechanisms by which plants perceive and respond to C6-volatiles is unknown.

To elucidate these mechanisms, we isolated several Arabidopsis mutants that do not respond to the C6-volatile E-2-hexenal. We refer to these mutants as hexenal-response (*her*) mutants. The mapping and characterization of one of these mutants, *her1*, identified GABA as a component of the E-2-hexenal response pathway, providing, for the first time, insight in the mechanism that Arabidopsis utilizes upon E-2-hexenal perception. The higher levels of GABA, which is predominantly present in the apoplast, in her1 results in a reduced susceptibility to *Pseudomonas syringae.*

Current work, involving the characterization and mapping of the *her2* mutant and the analysis of the E-2-hexenal induced transcriptome in wild-type and *her1* plants, is revealing additional components of the E-2-hexenal response pathway in Arabidopsis.

P316 Multiple hormone signaling sectors are shared among various types of inducible defense

Kenichi Tsuda, Masanao Sato1,2, Thomas Stoddard, Jane Glazebrook, Fumiaki Katagiri

1Dept of Plant Biol, Microbial and Plant Genomics Inst, Univ of Minnesota, St Paul, MN, USA, 2Dept of Life Sci, Grad Sch of Arts and Sci, Univ of Tokyo, Tokyo, Japan

The major signaling mechanisms for the pattern-triggered immunity (PTI) and the effector-triggered immunity (ETI) are not known due to the involvement of poorly characterized signaling pathway(s), functional redundancy among signaling pathways, or both. To test the possibility of functional redundancy we constructed an Arabidopsis quadruple mutant *dde2/ein2/pad4/sid2*. The level of PTI was tested using microbe-associated molecular pattern (MAMP)-induced resistance against *Pseudomonas syringae* pv. *tomato* DC3000 (Pto DC3000). In the quadruple mutant, 80% and 45% of flg22- and elf18-induced resistance, respectively, was lost. The level of ETI was tested by comparing the growth of the Pto DC3000 strains expressing the effectors, AvrRpt2, AvrRpm1, and AvrPphB to the growth of the Pto DC3000 carrying the empty vector. In the quadruple mutant, 80%, 20%, and 50% of ETI triggered by the effectors AvrRpt2, AvrRpm1, and AvrPphB, respectively, was lost. Furthermore, the quadruple mutant was more susceptible to a necrotrophic fungal pathogen, *Alternaria brassicicola*, than *dde2* or *pad3* single mutants. These results show that the signaling network defined by the four genes is mostly responsible for flg22-PTI and AvrRpt2-ETI and that the signaling network is shared among PTI, ETI, and resistance against a necrotroph. To precisely estimate the effects of single wild-type genes and their interactions, we measured flg22- and elf18-induced resistance, AvrRpt2- and AvrRpm1-ETI, and resistance against *A. brassicicola* in plants with all possible combinations of the four gene mutations and fitted a mixed general linear model to the obtained data. This signaling allocation analysis demonstrated that each of the four genes can positively contribute to resistance against both biotrophic and necrotrophic pathogens and that PTI and ETI have qualitatively different signaling allocation patterns that are quantitatively different between cases of the same type of resistance.

P317 Genetic analysis of salicylic acid perception

Juan Vicente Canet, Alejandra Roig, Albor Dobón, Pablo Tornero

IBMCP (UPV-CSIC)

Plants have developed defense mechanisms that are both complex and effective against pathogens. Salicylic acid (SA) synthesis and perception are crucial to some of them. This hormone induces the protein NPR1

(Nonexpresser of PR genes 1) to become an active monomer in the nucleus. There, NPR1 interacts with transcription factors, inducing the expression of PRs (Pathogenesis Related) genes.

Despite the importance of this defending path, only one mutant implicated in the SA perception has been described (*npr1*). This project originates from the hypothesis that other genetic components of the SA perception exist and have not been described yet.

But, to find them, it was necessary to design a method allowing to analyze a very high number of seeds, this being far from the traditional approach of symptoms associated to infection. To do so, a chemical analog to SA has been used: benzothiadiazole (BTH). This compound not only provokes an increased resistance to virulent pathogens but also a significant loss of growth measured as fresh weight. Furthermore, it does not have the problems of phytotoxicity and stability of SA.

Taking advantage of the loss-of-weight phenotype, we have calibrated a response to BTH that consists in four treatments during two weeks. To validate it, the levels of response to BTH of one hundred mutants relevant for defense or related signals have been quantified, as well as of the homologues, suppressors and interactors described for *npr1*. The conclusion is that only *npr1* alleles or new mutants (not described yet) would be obtained with such a screening.

The robustness and simplicity of this phenotype has allowed us to screen 4.4 million of mutagenized seeds to look for mutants that do not respond to BTH (*nrb*). As it was expected, most of these new mutants are *npr1* alleles. This, far from being bad news, will allow us to study this crucial protein with new tools. The distribution of the mutations in *NPR1* will be presented, along with a hypothesis that explains why is biased.

P318 The site of action of salicylate antagonism in the jasmonate signalling pathway

Antonio Leon-Reyes1, Dieuwertje Van der Does1, Annemart Koornneef1, Laurens Pauwels2, Hana Návarová1, Saskia Van Wees1, Elvira S De Lange1, Alain Goossens2, Johan Memelink3, Tita Ritsema1, Corné Pieterse1

1Utrecht University, Utrecht, the Netherlands, 2VIB, Ghent University, Ghent, Belgium, 3Leiden University, Leiden, The Netherlands

The plant hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) play a central role in plant defense. Their signaling pathways cross communicate, providing the plant with the capacity to finely tune the induced defense responses that are activated upon pathogen or insect attack. In Arabidopsis, several molecular players involved in the regulation of SA-JA cross-talk have been identified.[1,2] Here, we present our work on antagonistic effect of SA on JA signaling. Using a molecular-genetic approach, we demonstrated that JA biosynthesis, jasmonate ZIM-domain repressor proteins (JAZs) and the receptor Skip-Cullin-F-box (SCFCOI) complex are not essential for the antagonistic interaction between SA and JA signaling. In silico analysis of JA-responsive genes that are sensitive to SA-mediated suppression revealed that their promoters are significantly enriched with the GCC-box motif GCCGCC. Analysis of transgenic lines carrying four copies of the GCC-box fused to the GUS reporter gene revealed that the GCC-box is sufficient for JA-induced expression and SA-mediated suppression. Collectively, our results indicate that the suppressive effect of SA on JA signaling is targeted at the level of gene transcription.

1 Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S. and Van Wees, S.C.M. (2009). Networking by small-molecule hormones in plant immunity. Nature Chemical Biology, in press.
2 Leon-Reyes, A. *et al* (2009). Ethylene modulates the role of NPR1 in cross talk between salicylate and jasmonate signaling. Plant Physiology 149:1797-1809.

P319 Sensitive and fast laser-based techniques to monitor signalling molecules in Arabidopsis

Simona Cristescu, Frans Harren

Life Science Trace Gas Facility, Radboud University Nijmegen, the Netherlands

The availability of a broad knowledge about the model plant *Arabidopsis thaliana* allows researchers to develop new approaches and test new concepts efficiently before their application in other species. Next to the molecular tool, there is a considerable interest in the detection of minute quantities of signaling molecules such as ethylene (C_2H_4) and nitric oxide (NO) released by plants under different conditions. Trace gas detectors based on infrared laser spectroscopy are an excellent option for this. Traditional methods including gas chromatography, gas chromatography combined with mass spectrometry, or dispersive IR absorption techniques are known to be time-consuming, often not very specific, in many cases not sensitive enough and not reliable due to the required concentration steps.

Briefly, the laser-based detector consists of a laser and an absorption cell, in which the gas is detected. By measuring the light absorption in a gas sample at a large number of different wavelengths (frequencies) in the fingerprint region (2.5 and 11 microns), basically all compounds can, in principle, be identified and their individual concentrations can be measured.

During the years our group has developed various laser-based trace gas detectors which are presently accessible to researchers within EU supported Life Science Trace Gas Facility. Using these detectors, ethylene and nitric oxide released by plants, pathogens, etc. can be monitored non-invasively at and below the ppbv-level (1 ppbv = 1 part per billion volume) in a minutes down to seconds time scale and without incubation periods. Thanks to their features, the combination of such detectors with a flow-through system was proven to be ideal in revealing dynamic processes in (single) plant. To illustrate this, applications in monitoring ethylene and nitric oxide emissions in regulation the defense response upon pathogen and insect attack in Arabidopsis are presented.

P320 Transcript profiling of chitosan-treated Arabidopsis seedlings

Giovanni Povero1, Elena Loreti2, Chiara Pucciariello1, Donatella Di Tommaso3, Gianluca Di Tommaso3, Alberto Piaggesi3, Pierdomenico Perata1

1Plant Lab, Scuola Superiore Sant'Anna, Piazza Martiri della Liberte 33, 56127 Pisa, Italy., 2IBBA-CNR, Via del Borghetto 80, 56100 Pisa, Italy., 3Valagro spa, Zona Industriale, Atessa, Chieti, Italy.

In nature, plants can recognize potential pathogens, thus activating intricate networks of defence signals and reactions. Inducible defence is often mediated by perception of MAMPs/PAMPs elicitors, such as Oligogalacturonides (OGs), chitin and its derivative chitosan (a glucosamine polymer). In this study, we treated Arabidopsis seedlings with chitosan and carried out a transcript profiling analysis (GeneChip microarrays) in order to identify genes and transcription factors involved in chitosan elicitation. The results show that, among genes up-regulated by chitosan, members from the following families were over-represented: jasmonate and defense responsive genes, camalexin and lignin biosynthetic genes. Several *WRKY* and *MYB* domain transcription factors are also strongly induced by chitosan. Based on the microarray results, we selected chitosan-induced genes and transcription factors representative of different metabolic and defense pathways. Using a qPCR approach we analyzed the expression patterns of these genes in response to purified chitosan at different treatment times (2, 3, 6, and 9 hours after treatment). Since many chitosan-induced genes and TF are known to be responsive to *Botrytis cinerea* inoculation, chitosan may play a role in inducing protection from this necrotrophic fungi. We thus evaluated the ability of chitosan to confer resistance from *Botrytis cinerea* in Arabidopsis adult leaves. The results suggest that chitosan can be used as a strong elicitor of defense pathways.

P321 DIACYLGLYCEROL KINASE 5 is required for SA responsiveness and disease resistance in Arabidopsis

Saskia C M Van Wees1, Bas Van Schooten2, Corné M J Pieterse1, Teun Munnik2

1Plant-Microbe Interactions, Institute of Evolutionary Biology, Utrecht University, Utrecht, the Netherlands, 2Plant Physiology, Swammerdam Institute for Life Sciences, University of Amsterdam, Amsterdam, the Netherlands

Upon elicitation by pathogen-derived elicitors, phosphatidic acid (PA) is produced which is proposed to function as a lipid second messenger. PA is generated via either the phospholipase C (PLC)/diacylglycerol kinase (DGK) or the phospholipase D (PLD) pathway. Using Arabidopsis *dgk5* knock-out mutants, we provide genetic evidence that DGK5 is required for resistance to the virulent pathogens *Pseudomonas syringae* pv *maculicola (Psm)* and Hyaloperonospora arabidopsidis Waco9. After *Psm* infection, *DGK5* gene expression was elicited within 6 hours, reaching 5-fold induction levels. Expression analysis of the salicylic acid (SA) signaling mutants *pad4*, *sid2*, and *npr1* showed that the induction of *DGK5* by *Psm* was not affected in these mutants. However, the resistance defect of *dgk5* was correlated with a strongly reduced expression of the SA-regulated *PR-1* gene after *Psm* infection. Upon treatment with SA the *PR-1* induction in *dgk5* was abolished as well, suggesting that DGK5 functions downstream of or in parallel with SA in defense signaling. In accordance, SA accumulated to similar levels in *dgk5* and wild-type plants upon *Psm* infection. However, the induction levels of SA-regulated genes like *PR-2, PR-5*, and *GRP94* were unaffected by the *dgk5* mutation, indicating that expression of only a subgroup of SA-dependent defense responses is affected in *dgk5*. Examination of the effect of DGK5 on functioning of NPR1, a key regulator of SA-dependent signaling, revealed that DGK5 does not affect translocation of NPR1 to the nucleus and thus may affect SA-regulated responses independently of NPR1.

P322 SSV1 and ATL6 as C/N regulatory E3 ligase, are also involved in immune response system in Arabidopsis

Shugo Maekawa1, Takeo Sato1, Yutaka Asada1, Shigetaka Yasuda1, Midori Yoshida2, Junji Yamaguchi1

1Hokkaido University, Sapporo, Japan, 2NARC Hokkaido, Sapporo, Japan

Protein degradation by ubiquitin/26S proteasome system (UPS) is involved in various aspects of cellular activity. Selection of target protein in the UPS is catalyzed by ubiquitin ligase (E3). In higher plants, metabolism of sugar (C) and nitrogen (N) is competitively regulated. To clarify the C/N regulator, we isolated novel C/N regulatory E3 ligase SSV1. The *SSV1* gene belongs to the ATL family. Although some ATLs have been reported to be involved in plant immunity, the detailed mechanisms are still unknown. To investigate the relationship between the ATL family and pathogen resistance, we examined pathogen resistance using *Pseudomonas syringae* pv. *tomato* DC3000. Plants overexpressing *SSV1* and *ATL6*, which is most closely related gene to the *SSV1* in ATL family, demonstrated an increase in pathogen resistance, whereas a decrease was observed in the *ssv1 atl6* double mutant. Relationships between C/N balance regulation and plant immunity will be discussed in terms of functions of the ATL family.

P323 Three *Hyaloperonospora arabidopsidis* RXLR effector proteins interact with members of the Arabidopsis prenylated Rab acceptor PRA1 family

Jens Steinbrenner, Mary Coates, Susan Donovan, Tina Payne, Peter Bittner-Eddy, Sarat Bimanadham, Volkan Cevik, Jim Beynon

Warwick HRI, Wellesbourne, University of Warwick, UK

An open question is how microbial pathogens manipulate the plant immune system to establish disease. Oomycete effectors have been reported to be delivered to the host cell to alter host immunity. Despite the fact that the primary sequence of these effectors is distinct, they have in common a signal peptide for secretion from the pathogen, followed by the motif RXLR and an acid region often

ending in the sequence EER, that enables entry into the host. Bioinformatic analysis of the *Hyaloperonospora arabidopsidis* genome revealed over 200 candidate RXLR effector genes. We have used yeast two hybrid screens, using a small number of RXLRs as baits, to identify interacting proteins from Arabidopsis. Three RXLRs identified members of the PRA1 family as potential protein targets. PRAs are small transmembrane proteins that play a role in the regulation of vesicle trafficking. Altering plant secretory pathways would be a logical target for pathogenicity effectors and we will describe confirmation of the interaction *in planta* and consequences of the presence of the RXLR proteins to the host.

P324 Functional analysis of differentially expressed proteins of *Arabidopsis thaliana* after infection with the pathogenic fungus *Verticillium longisporum*

Christine Druebert, Saskia Floerl, Andrea Polle

Buesgen Institute, Goettingen, Germany

Verticillium longisporum is a soil born pathogen with agricultural relevance that colonizes the xylem of Brassicaceae. Also, *Arabidopsis thaliana* shows symptoms after infection with *Verticillium longisporum*, namely reduced leaf area, yellowing of the leaves and pre-mature senescence. The physiological changes and defence reactions that occur within the plant after infection are mainly unknown. Since the fungus is located in the xylem the main defence effects are expected in the extracellular compartment. Therefore, the extracellular proteome of infected and control Arabidopsis plants was analysed. Seven proteins were differentially expressed after *Verticillium longisporum* infection. To functionally characterise these proteins knock out mutants and overexpressing plants are tested for enhanced susceptibility to *Verticillium longisporum*. Fresh weight, leaf area and chlorophyll content are used for evaluation of susceptibility. Furthermore, the amount of *Verticillium longisporum* within the plant material is analysed by real time PCR. First results of the investigated knock out lines will be presented.

Financial support by the DFG (Forschergruppe 546) is gratefully acknowledged.

P325 Functional analysis of transcriptional repressors DEAR1 and ERF9 in terms of plant defense in Arabidopsis

Yutaka Asada, Natsuko Yamoto, Kazuma Takahashi, Kuniaki Katsumata, Junji Yamaguchi

Graduate School of Life Science, Hokkaido Univ, Sapporo, Japan

The *DEAR1* (DREB and EAR motif protein 1), which encodes the protein containing DREB domain and EAR motif, is likely to function as a transcriptional repressor to the DRE-binding protein. The *DEAR1* overexpressor showed constitutive cell death on rosette-leaves and accelerated resistance to necrotrophic fungi *Botrytis cinerea.* The overexpressor also stimulated expression of *PDF1.2* gene, which is controlled by *ERF* (ethylene-responsive element binding factor) genes. DNA microarray and expression analysis on *DEAR1* overexpressor revealed that ERF9, which contains the EAR motif and has a DRE region in the promoter, is the target of *DEAR1*. It is likely that the ERF9 usually represses expression of target genes including *PDF1.2* and that releases the repression by the *DEAR1* at pathogen infection. The *ERF9* gene under control by the *DEAR1* will be discussed.

P326 Systemic disease resistance conferred by the mycorrhiza fungus *Piriformospora indica* requires jasmonic acid signaling and the cytoplasmic function of NPR1

Alexandra Molitor1, Elke Stein1, Karl-Heinz Kogel1, Frank Waller1,2

1Institute of Phytopathology and Applied Zoology Justus Liebig University Giessen, 35392 Giessen, Germany, 2present address: Julius-von-Sachs-Institute, Julius-Maximilians-University Wuerzburg, Wuerzburg, Germany

The beneficial basidiomycete *Piriformospora indica* colonises the roots of plants of different phylogenetic groups, including *Arabidopsis thaliana* and crop plants like barley and rice. In barley, *P. indica* colonised plants are more resistant against the biotrophic powdery mildew fungus *Blumeria graminis f.* sp. *hordei*, and show a higher growth rate and increased grain yield. In Arabidopsis, root colonisation by *P. indica* leads to systemic resistance against the powdery mildew fungus *Golovinomyces orontii.*

P. indica root colonization induced a reduction of *G. Orontii conidia* in wild type (Col-0), as well as in *nonexpressor of PR1-3 (npr1-3)* and NahG plants, but not in the *npr1-1* null mutant. As *npr1-3* is lacking the nuclear localisation signal, the cytoplasmic function, but not nuclear localization of NPR1 is required for *P. indica*-induced resistance. In addition, *jasmonic acid resistant (jar1-1)* and *jasmonate-insensitive 1 (jin1)* were compromised in resistance induction by *P. indica*, indicating that components of the jasmonic acid signalling pathway are also required. Consistently, jasmonic acid-responsive vegetative storage protein 1 expression was primed and thus elevated in response to powdery mildew.

In conclusion, induction of pathogen resistance by *P. indica* root mycorrhization requires similar pathways as induced systemic resistance (ISR) described for root-colonising Rhizobacteria.

P327 The WRKY33 transcription factor of Arabidopsis is involved in balancing the hormone signaling pathways and adjusting the metabolic response to Botrytis infection

Rainer Birkenbihl, Imre Somssich

Max Planck Institute for Plant Breeding Research, Cologne, Germany

Plants possess very effective defense mechanisms to protect themselves against a wide range of microbial pathogens. These mechanisms include the recognition of pathogens followed by signaling to the nucleus, where transcription factors are activated and modulate expression to appropriately adjust the plant's metabolic

response. WRKY proteins, constituting a large transcription factor family, play a vital role in this process. They are individually involved in the regulation of responses to different biotic and abiotic stresses. WRKY33 is especially involved in regulating the response towards necrotrophic fungi like *Botrytis cinerea.* While wild type Arabidopsis Col-0 plants are nearly resistant to this fungus, the *wrky33* KO mutant is highly susceptible. The molecular basis for this is still widely unknown. By comparing *wrky33* mutant and wild type plants before and after treatment with *B. cinerea* spores, we show on the transcript and metabolite levels that the response to the developing fungus is highly complex and that distinct differences between wild type and the mutant exist. In the mutant, the balance between the two antagonistically acting defense signaling pathways, the one mediated by salicylic acid in response to biotrophic pathogens, and the other mediated by jasmonic acid in response to necrotrophic pathogens, is particularly disturbed. In the *wrky33* mutant both hormones accumulate to higher levels than in wild type plants. As a consequence of this, transcript levels of specific SA- and JA-dependent genes differ significantly from wild type. Additionally, genes encoding key enzymes acting in the synthesis of defense-related metabolites are mis-regulated. One clearly measurable consequence of this is that the level of camalexin, a broad-spectrum phytotoxin, is substantially reduced in the mutant, which by itself may already promote growth of this particular fungus.

P328 Involvement of autophagy in plant defense

Heike D Lenz, Andrea A Gust, Thorsten Nürnberger

Department of Plant Biochemistry, Center for Plant Molecular Biology, University of Tübingen, Germany

The autophagic cascade was primarily identified as a cellular response to starvation to survive periods of nutrient deficiency. Autophagy has further been implicated in disposal of protein aggregates, removal of damaged proteins and organelles during normal growth conditions, senescence and oxidative stress, but also in the regulation of programmed cell death. In *Arabidopsis thaliana*, it was shown that plants lacking ATG6, which is important for autophagy, were impaired in the restriction of programmed cell death after treatment with incompatible *Pst* DC3000 bacteria containing the AvrRpm1 effector protein.[1]

In this project, we investigate the role of autophagy-associated genes (ATGs) in the plant innate immune response. Database searches based on homology to yeast revealed 33 ATG genes in *Arabidopsis thaliana*. In microarray analysis, 13 of these 33 genes, such as *ATG7* and *ATG18* were transcriptionally upregulated upon pathogen infection. For these induced genes and additional genes playing a proven role during the conjugation cascades, such as *ATG5* and *ATG10*, we collected T-DNA insertion and RNAi lines to silence whole gene families. Knock out lines were analysed with respect to their defense response after infection with different Pseudomonas syringae strains or necrotrophic fungi, like *Alternaria brassicicola* and *Botrytis cinerea*. We will present data for *atg5*, *atg7*, *atg10* and *atg18* knock-out lines, which show an increased resistance to Pseudomonas infection, but a decreased resistance to necrotrophic fungi compared to wild type plants. Moreover, responses to cell death-inducing elicitors such as NLP and Fumonisin were tested. In addition, we also examined responses to different abiotic stress conditions, such as osmotic, salt or drought stress in mutant plants in comparison to wild type plants.

Patel and Dinesh-Kumar, Autophagy 4:1, 20-27, 2008

P329 The oxidative pentose phosphate pathway and plant defense responses

Zhonglin Mou, Yuqing Xiong, Christopher DeFraia, Donna Williams, Xudong Zhang

Department of Microbiology and Cell Science, University of Florida, Gainesville, FL, USA

The inducible defense mechanism systemic acquired resistance (SAR) requires the signal molecule salicylic acid (SA) and the positive regulator NPR1. SAR induction changes the redox state of plant cells, leading to reduction of cytosolic NPR1 oligomer to monomers. Monomeric NPR1 accumulates in the nucleus and activates defense gene expression. The oxidative pentose phosphate pathway (oxPPP) has been proposed to contribute to the SAR-related redox changes. However, genetic evidence supporting this hypothesis is still lacking. Here we show that, similar to SA treatment, knockdown of the plastidial 6-phosphogluconolactonase (PGL3), which is essential for plant growth and development, increases the activity of the oxPPP key regulatory enzyme glucose-6-phosphate dehydrogenase (G6PD) and changes the redox equilibrium of plant cells to a more reduced state. In *pgl3*, NPR1 exists in both oligomeric and monomeric forms, and constitutively accumulates in the nucleus. The *pgl3* plants also exhibit constitutive pathogenesis-related (PR) gene expression, and display enhanced resistance to *Pseudomonas syringae* pv. *maculicola* ES4326 and Hyaloperonospora parasitica Noco2. Both PR gene expression and pathogen resistance of the *pgl3* plants are significantly suppressed by the *npr1* mutation. Although *pgl3* does not spontaneously accumulate elevated levels of free SA, the PR gene expression and pathogen resistance of the *pgl3* plants can also be significantly suppressed by the *sid2/ics1* mutation. These results suggest that *pgl3* activates NPR1- and SID2/ICS1-dependent defense responses. Taken together, our results indicate a tight correlation among a genetic mutation in the oxPPP, redox change, and activation of defense responses.

P330 Analysis and comparison of regulatory mechanisms of *PR-4* genes in Arabidopsis and wheat

Silvia Proietti1, Sjoerd Van der Ent2, Antonio Leon-Reyes2, Corné M J Pieterse2, Laura Bertini1, Carlo Caporale1, Carla Caruso1

1Department of Agrobiology and Agrochemistry Tuscia University, Viterbo, Italy, 2Plant-Microbe Interactions Institute of Environmental Biology Utrecht University, Utrecht, The Netherlands

Throughout their life-time plants are continuously attacked by pathogenic micro-organisms that aim to exploit the

plant's assimilates as a food source. Therefore, plants strongly rely on their ability to recognize these attackers and subsequently activate defense responses that are effective against the invader encountered. One such defense response is the pathogen-induced accumulation of PR (PATHOGENESIS RELATED) proteins in both local and, to a less extent, systemic plant tissues. Based on sequence similarity between the encoding genes, we recently isolated *wPR4e*, the wheat *(Triticum aestivum)* ortholog of Arabidopsis *PR-4/HEL*. The gene encoding the former protein, *wPR4e*, exhibited similar expression patterns upon pathogen infection, chemically induced systemic acquired resistance, and wounding, as Arabidopsis *PR-4/HEL*. These results suggest functional conservation of the PR-4 orthologs between the dicot, Arabidopsis, and the monocot, wheat. Moreover, they might imply similarities in the transcriptional regulation of *wPR4e* and *PR-4/HEL*. Indeed, *in silico* analysis of *wPR4e* and *PR-4/HEL* promoter sequences revealed the presence of several similar cis-acting motifs, which serve as docking sites for regulating transcription factors. Among these were W-boxes that allow DNA-binding by stress-related WRKY transcription factors. Interestingly, we also identified a wheat WRKY (TaWRKY1), which is orthologous to Arabidopsis AtWRKY20. Expression analysis of the two corresponding genes showed that they are responsive to the same stimuli. Furthermore, we demonstrated that both TaWRKY1 and AtWRKY20 act as transcriptional activators of *wPR4e* and *PR4/HEL*, respectively. Moreover, we demonstrated the effect of TaWRKY1 on *PR-4/HEL* expression as well as the effect of *AtWRKY20* on *wPR4e* expression.

Hence, on the basis of these results we have evidence that Arabidopsis and wheat seem to use similar regulatory mechanisms to activate PR-4 genes.

P331 A family of ubiquitin ligases transmits ROS signals in plant cell death induction and stress response

Karolin Eifler1,2, Wolf-Rüdiger Scheible3, Nikolaus Schlaich4, Dieter Ernst5, Maria Novatchkova6, Robert Konrat7, Marcus Garzón2, Kerstin Luxa2, Andreas Bachmair1,2

1Univ. of Vienna, Max F. Perutz Laboratories, Vienna, Austria, 2Max Planck Institute for Plant Breeding Research, Cologne, Germany, 3Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany, 4RWTH Aachen, Aachen, Germany, 5Helmholtz Zentrum München, Neuherberg, Germany, 6Research Institute of Molecular Pathology, Vienna, Austria, 7Univ. of Vienna, Dept. of Biomolecular Structural Chemistry, Vienna, Austria

Ubiquitin is a small modifier protein that can be covalently linked to substrates via a dedicated set of enzymes, called ubiquitin activating enzyme E1, ubiquitin conjugating enzymes E2 and ubiquitin ligases E3. Ubiquitin conjugation plays a pivotal role in virtually every hormonal response. In animals, it is important in diverse stress reactions and in cell death programs. Similar functions in plants are, however, only poorly defined. We are studying a group of ubiquitin ligases with an apparent role in plant cell death processes. Plants with mutation in some of these genes are more resistant to normally lethal stimuli caused by ozone exposure, and by a nitric oxide donor. The ubiquitin ligases can be divided into two classes. One class localizes to the cytoplasm and has homologs in animals and some fungi, whereas the other class is apparently plant-specific and localizes mainly to the nucleus (with low presence in the cytoplasm). In the yeast two hybrid system, members of one group interact with the other group, but both classes can also form homo-dimers. We speculate that both types of ligases are part of a signal transduction cascade from the cytoplasm to the nucleus, thereby transmitting ROS and NO signals.

P332 Additive effects of RNAi-mediated plant resistance against root knot nematodes

Wayne L Charlton, H Yael Meller Harel, Manjula Bakhetia, Jennie K Hibbard, Howard J Atkinson

University of Leeds, Leeds, UK

Ectopically expressed dsRNA has recently been shown to suppress parasitic success of *Meloidogyne* spp. in plants. We have targeted two genes from the root-knot nematode *Meloidogyne incognita*; a dual oxidase gene implicated in the tyrosine cross-linking of the developing cuticle and a subunit of signal peptidase, a protein complex required for the processing of secreted proteins. While these genes are involved in different aspects of nematode development, the phenotypic consequences of RNAi were similar with ≥50% reduction in nematode numbers in the roots and retardation of development to the reproductively important saccate female stage. Combining expression of these dsRNAs by crossing appropriate *Arabidopsis thaliana* lines resulted in an additive effect further reducing nematode numbers and developmental capacity. This is consistent with the probable temporally distinct expression patterns of the genes. Combining RNAi target genes has the potential to enhance the efficacy of RNAi and may allow control of different nematode species or genera.

P333 The *Arabidopsis thaliana* protein phosphatase PP2C5 is a novel MAPK phosphatase regulating ABA signalling

Anita Brock1, Roland Willmann1, Laure Grefen1, Dagmar Kolb1, Irute Meskiene2, Alois Schweighofer2, Thorsten Nürnberger1, Andrea Gust1

1Department of Plant Biochemistry, Centre of Molecular Plant Biology, University of Tübingen, Tübingen, Germany, 2Max F. Perutz Laboratories of the University of Vienna, Vienna, Austria

Abscisic acid (ABA) is an important phytohormone regulating various cellular processes in plants, including stomatal opening and seed germination. Although protein phosphorylation via mitogen-activated protein kinases (MAPKs) has been suggested to be important in ABA signalling, the corresponding phosphatases are so far elusive. Here we show that a member of the PP2C family in Arabidopsis, PP2C5, is acting as a MAPK phosphatase. The PP2C5 protein co-localizes and directly interacts with stress-induced MPK3, MPK4 and MPK6 predominantly in

the cell nucleus. Importantly, PP2C5 protein levels affect MAPK activation. Whereas *pp2c5* knock out plants showed an enhanced stress-induced activation of MAPKs, ectopic expression of PP2C5 had the opposite effect. Moreover, depletion of PP2C5, whose gene expression itself is affected by ABA treatment, resulted in altered ABA responses. Loss-of-function mutation in *PP2C5* as well as *AP2C1,* a close PP2C5 homolog, resulted in an increased stomatal aperture under normal growth conditions and a partial ABA insensitive phenotype in seed germination which was most prominent in the *pp2c5/ap2c1* double mutant line. In contrast, ABA-inducible genes were constitutively up-regulated in the double mutant. Thus, we present PP2C5 as the first phosphatase that acts as MAPK phosphatase and negatively regulates ABA-inducible gene expression while at the same time positively regulating seed germination and stomatal closure.

P334 Effect of the geminivirus C2/L2 protein on the CSN complex

Rosa Lozano-Durán1, Alberto P Macho1, Giuliana Gusmaroli2, Ana P Luna1, Adela Zumaquero1, Carmen R Beuzón1, Xing Wang Deng2, Eduardo R Bejarano1

1University of Malaga, Spain, 2MCDB Yale University, New Haven CT, United States

Geminiviruses are a family of plant viruses with highly reduced DNA genomes, encoding for only 6 to 8 proteins. To accomplish the infection, geminiviruses must create a suitable cell environment, which most likely involves interaction with the cellular machinery and, ultimately, entails changes in the cellular homeostasis. These modifications of the cellular state will allow the virus to profit from the host cell machinery for its vital functions and to elude plant defence mechanisms.

C2/L2 is a multifunctional geminiviral protein which has been described as a transcriptional activator, for both plant and viral genes, and as a gene silencing suppressor in some geminivirus species. However, little is known about the molecular mechanisms underlying C2 activities. Previous data obtained in our lab suggest that C2 has an important role in the infection process.

We have found that C2 of at least three different geminiviruses interact with Arabidopsis CSN5A, catalytic subunit of the CSN complex, using a yeast two hybrid assay. The interaction between a viral protein and the host cell CSN complex has never been described for any virus, neither plant nor animal, before. Moreover, the activity of the CSN over CULLIN1 seems to be compromised in transgenic Arabidopsis lines expressing C2.

The response to some plant hormones is altered in these Arabidopsis transgenic lines expressing C2: they show differential sensitivity to jasmonates and the bacterial toxin coronatine, 2,4-D, gibberellins, ACC, and ABA in the guard cells. All these processes are regulated by SCF complexes, which are CULLIN1-based E3 ubiquitin ligases. These data suggest that C2 might be affecting the activity of this major class of E3 ligases, most likely through the partial inhibition of the CSN.

Taking into consideration that SCFs are key regulators of many cellular processes, the capability of Geminiviruses to selectively interfere with or hijack the activity of these complexes might mean a novel strategy in virus infection.

P335 Plant SUMO paralogs have distinct functions in development and innate immunity

Harrold A van den Burg1,2, Ramachandra Kini2, Pierre J G M de Wit1, Frank L W Takken2

1Laboratory for Phytopathology, Wageningen University, the Netherlands, 2Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, the Netherlands

SUMO (small ubiquitin-like modifier) is a post-translational modification that modulates the activity and the recruitment of proteins to transcription complexes. In Arabidopsis abiotic stress (e.g. heat shock and dehydratation) induce massive conjugation of SUMO to an apparently large set of substrates. The reason for this mass SUMOylation remains obscure and no comprehensive identification of these substrates has yet been undertaken leaving their identity unknown. We study the redundant and non-redundant biological functions of the different SUMO paralogs using TDNA insertion lines and dominant-negative mutants. These mutants show that SUMO paralogs differentially regulate plant development and salicylic-acid dependent innate immunity. Our data indicate that in plants SUMO paralogs have acquired different functions in three ways: (1) differential gene induction and spatial expression patterns, (2) sequence differences promote paralog-specific interactions, and (3) the capacity to form poly-SUMO tails. We now want to identify SUMO substrates and interactors using a proteomics approach and a targeted-screening approach.

HvdB kindly acknowledges Netherlands Scientific Organization NWO-VENI grant).

P336 Identification of PAMPs from *Xanthomonas axonopodis* pv *citri*

Martin Lipschis, Georg Felix

Institute of Plant Biochemistry, ZMBP, University of Tuebingen, 72076 Tuebingen, Germany

A conserved aspect of active immune responses in multicellular organism is the ability to sense microbial invaders via perception of pathogen-associated molecular patterns (PAMPs). The corresponding pattern recognition receptors (PRRs) include Toll and Toll like receptors in animals and receptor kinases and receptor like proteins in plants. In previous work we identified the leucine rich repeat receptor kinases (LRR-RKs) FLS2 and EFR in Arabidopsis as the pattern recognition receptor for the bacterial PAMPs flagellin and EF-Tu. Interestingly, mutants lacking these receptors still respond to bacterial preparations with induction of typical PAMP responses such as rapid extracellular alkalinization and increased ethylene biosynthesis. LPS and PGN, bacterial PAMPs reported to be active in Arabidopsis, did not induce comparable responses in these biosassays, indicating

perception systems for further, unknown bacterial PAMPs in Arabidopsis. Here, we started to purifiy and analyze such a novel PAMP from *Xanthomonas axonopodis* pv. *citri (XAC).* While living bacteria induced defense reactions, activity was much stronger in the supernatant of sonicated bacteria. The activity in these XAC preparations was found to be heat labile and sensitive to treatment with proteinase K and trypsin, indicating association with a proteinaceous structure. So far, only partial purification of this activity has been achieved by ion-exchange- and hydrophobic interaction-chromatography. While active on *A. thaliana*, XAC fractions were inactive in leaves and/or cell cultures of *N. benthamiana, L. peruvianum* and *O. sativa*. Thus, in contrast to PAMPs like the flg22-epitope of flagellin which is recognized by a broad variety of plant species, the perception systems for this novel PAMP might exist in a more narrow range of species only. Examples for PAMPs perceived by a limited range of plant species also include EF-Tu, perceived by Arabidopsis and closely related plants but not in species outside the *Brassicales*, and the RNP-1 motif of bacterial cold shock proteins that acts as a PAMP in species of the *Solanales* but not in the *Brassicales*.

P337 Keeping up multiple biotic stress responses

Mieke de Wit1, Sjoerd van der Ent2, Laurentius ACJ Voesenek1, Ronald Pierik1

1Plant Ecophysiology, Institute of Environmental Biology, Utrecht University, Sorbonnelaan 16, 3584 CA, Utrecht, The Netherlands, 2Plant-Microbe Interactions, Institute of Environmental Biology, Utrecht University, Sorbonnelaan 16, 3584 CA, Utrecht, The Netherlands

Plants in dense stands have to compete for light. Upon neighbour detection, a suite of responses called the shade avoidance syndrome is induced within the plant to increase light interception. Apart from the possibility of becoming out-grown by other plants, growth in high densities brings along an increased risk of pathogen attack. Neighbouring plants in close proximity facilitate plant-plant infection and the enclosed atmosphere of a canopy forms a microclimate favourable for pathogens. To defend themselves against pathogens, plants possess several preformed and inducible defense mechanisms. Both the shade avoidance and pathogen defense responses have been studied extensively in isolation. How plants cope with simultaneous stress from both competitors and pathogens however remains largely unknown. Through physiological responses and marker gene expression we try to gain insight into how plants can compete and defend themselves against pathogens at the same time.

We show that shade avoidance induction through a combination of light signals suppresses pathogen defense, tentatively through down-regulation of salicylic acid-mediated defense routes. In competition experiments using dense Arabidopsis populations, however, interactions may be more complex, thereby affecting both competitive strength and defense. Early results and future directions will be discussed.

P338 Hunting jasmonate targets with yeast three-hybrid technology

Stephanie Cottier1, Katja Schneider1, Zheming Wang2, Markus Kaiser2, Erich Kombrink1

1Max Planck Institute for Plant Breeding Research, Department Plant-Microbe Interactions, 50829 Köln, Germany, 2Chemical Genomics Centre, 44227 Dortmund, Germany

Multicellular organisms, whether plant or animal, coordinate their growth and development and many responses to the environment by using small signalling molecules for communication between cells and organs. Among the established plant hormones, jasmonates (JAs) play a role in plant immunity and different developmental aspects. Recent findings elucidated the jasmonic acid-isoleucine (JA-Ile) signal transduction, which acts trough the formation of a ternary complex involving JA-Ile, COI1 and JAZs. However, mechanisms responsible for COI1-independent JA responses and the possible bioactivity of the other jasmonic acid derivatives is still an open question.

We initiated a search for putative JA targets by using the yeast three-hybrid technology. This new experimental approach allows direct cloning of protein that interacts with synthetic hybrid ligands *in vivo*. Such hybrid ligands consist of methotrexate coupled via a PEG spacer to various jasmonic acid derivatives, as well as coronatine, a bacterial phytotoxin mimicking JA-Ile. We used different cDNA libraries to screen a large part of the Arabidopsis expressed genome. Here we show that yeast three-hybrid technology is a valid approach to generate JA-binding candidates. Although the biological significance of these interactions remains to be established, yeast three-hybrid screens show great promise for the isolation of small signalling molecule targets in plants, and could potentially be extended to other hormones or phytotoxins.

P339 Cloning *ATR5* from Arabidopsis downy mildew pathogen *Hyaloperonospora arabidopsidis*

Kate Bailey, Volkan Cevik, Nick Holton, Eric Holub, Mahmut Tor

Warwick HRI, Warwick, UK

Understanding the mechanisms of microbial pathogenesis and plant-microbe interactions has motivated plant pathologists for a long while. Microbe-associated molecular pattern molecules (MAMPs) and effector molecules have been found to perform inter- and intracellular tasks as adaptation factors and manipulators of the defence network. The *Arabidopsis-Hyaloperonospora* pathosystem has played a significant role in uncovering major complementary AVR-R genes. Cloning of *ATR1* and *ATR13* from *H. arabidopsidis* (formerly *H. parasitica*) and others including *Avr1b-1* and *Avr3a* from Phytophthora species has enabled the identification of common conserved regions including the N-terminal RXLR and EER motifs. Arabidopsis La-er accession carries *RPP5*, which recognizes *ATR5* from Noks1/Noco2 and Emoy2 isolates. We have been carrying out map-based cloning of *ATR5*

using F2 mapping populations derived from different crosses between isolates of *H. arabidopsidis.* A genetic interval for *ATR5* has been established and a physical map of *ATR5* was constructed using the publicly available genomic and BAC-end sequences, as well as the BAC contig data. Further delineation of the *ATR5* locus was carried out and the gene has been placed on a single BAC clone. Fine mapping has put the gene to a 25kb interval. Bioinformatic studies supported by expression analysis revealed the presence of five genes, three of which have the characteristics of effector molecules. Interestingly, none of these candidates have an RXLR motif. Transient expression studies using bombardment assays have identified *ATR5* among these candidates and demonstrated it to give an *RPP5* dependent defence response. Recent work on the function, evolution and further analysis will be presented.

P340 Structural and functional analysis of the type III secretion system effector AvrRpm1

Karen Cherkis1, Jeffery Dangl1, John Sondek2

1University of North Carolina Curriculum in Genetics and Molecular Biology, Chapel Hill, (NC), USA, 2University of North Carolina Department of Pharmacology, Chapel Hill, (NC), USA

Pseudomonas syringae utilizes a type III secretion system (TTSS) to deliver type III effector proteins (virulence factors) into the host cell. These effectors can elicit susceptibility or resistance depending upon the host. In a disease susceptible plant the host succumbs to infection and the pathogen is allowed to propagate. In a disease resistant plant the pathogen is directly perceived via pathogen-associated molecular patterns (PAMPs), thereby triggering basal defense responses such as callose deposition. If the pathogen bypasses this first line of defense, plant resistance proteins (R proteins) can perceive the bacterial effector(s) and initiate downstream defense responses such as the hypersensitive response (HR), a type of cell death. This perception is typically mediated via perturbation of a host protein acting as an intermediate. AvrRpm1 is a type III effector from *Pseudomonas syringae* pv. *maculicola*. Upon secretion into the host cell, AvrRpm1 can interact with and directly or indirectly cause phosphorylation of Arabidopsis RIN4. This perturbation of RIN4 is perceived by the R protein RPM1 in resistant plants. The exact mechanism employed by AvrRpm1 has yet to be elucidated.

We are investigating AvrRpm1 by adopting a structure-function approach. Using homology modeling, we identified AvrRpm1 to have a similar fold to poly-ADP-ribosylpolymerase (PARP). We have carried out site-directed mutagenesis of the putative catalytic triad His-Tyr-Asn in AvrRpm1 to determine whether AvrRpm1 has ADP-ribosylation activity or is a structural mimic of the host PARP(s). Furthermore, we are purifying AvrRpm1 for future crystallization trials to determine its native structure.

P341 Control mechanisms for activation of a novel CC-NBS-LRR protein, UNI-mediated signals that induce both SA-dependent defense and CK-dependent morphological signals

Kwi Mi Chung, Kadunari Igari, Masao Tasaka

Nara Institute of Science and Technology, Ikoma, Japan

We have previously described the Arabidopsis semi-dominant mutant *uni-1D*, in which heterozygotes display bushy, severe dwarf phenotypes, forming many narrow leaves and inducing many ectopic axillary meristems; homozygotes die after a few abnormal leaves have been formed. The gene responsible encodes a coiled coil-nucleotide binding site-leucine rich repeat (CC-NBS-LRR)-type protein belonging to the R gene family, whose members are involved in pathogen recognition and resistance. Its mutation causes salicylic acid (SA)-dependent pathogenesis-related gene expression, and it also induces cytokinin (CK)-mediated morphological defects. In this study, we focused on how *uni-1D*-mediated signals can be activated in transcriptional and proteomic levels. The phenotypes which were shown in *uni-1D* mutant could be induced by overexpression of genomic wild-type UNI DNA under its own promoter. These data indicated that *uni-1D*-mediated signals are dependent not only on the existence of its mutation but also on dosage of wild-type UNI. Both mutant (*uni-1D*) and *wild type (wt) UNI* produce two splicing variants. Analysis of transgenic plants expressing individual splicing variants indicated that in the case of *wt UNI*, *uni-1D* like phenotype can be induced only when its spliced form was highly expressed, on the other hand, that for *uni-1D*, signals can be activated by just the presence of its spliced form. To identify proteins involved in UNI-mediated signals, we have performed yeast two-hybrid screens and isolated several candidate UNI-interacting proteins. Genetic and biochemical studies about the relationship between UNI and these proteins are underway.

P342 A proteomic approach to discover biosynthetical and regulatory protein complexes in plant secondary metabolism

Jan Geerinck, Maarten Dedecker, Eveline Van de Slijke, Geert Persiau, Yelle Buffel, Dominique Eeckhout, Laurens Pauwels, Sofie Tilleman, Dirk Inzé, Geert De Jaeger, Alain Goossens

VIB Department of Plant Systems Biology, Ghent University, Belgium

Plants are known to produce an enormous variety of secondary metabolites. Although these compounds are not strictly essential for growth, they play a pivotal role in the interaction of the plant with its environment and very often contribute to its survival. As the production of secondary metabolites is energy demanding, it is clear that these processes need to be very well controlled, both on the regulatory and the biosynthetical level.

Our research focuses on two such processes: the jasmonate signalling cascade and the monolignol biosynthesis pathway. Jasmonates are known elicitors of plant secondary metabolism, capable of inducing a vast

array of different compounds. Lignin, on the other hand, is the most abundant polymer in the plant kingdom and its biosynthetical pathway is well studied.

To gain deeper insight in these two events and to discover potentially new players involved, we use proteomics tools such as Blue Native PAGE and Tandem Affinity Purification (TAP) to investigate protein complex presence and formation involved in these processes. We were capable of demonstrating the dynamic interplay of MYC2, JAZ1 and COI1 under differential experimental conditions. Furthermore, we show that BN-PAGE and TAP-MS are appropriate techniques to study protein interactions in monolignol biosynthesis.

P343 The SUMO protease OTS1 is a novel component affecting the SA-mediated response

Lucio Conti1, Andrew Love1, Elisabeth O'Donnell1, Byung-Wook Yun2, Gary Loake2, Joel Milner1, Ari Sadanandom1

1FBLS, University of Glasgow, Glasgow, UK, 2Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh, UK

Resistance to biotrophic pathogens in plants is dependent on pathogen-triggered elevations in salicylic acid (SA). This induces the expression of a plethora of antimicrobial genes, leading to pathogen destruction. Although SA level modulation is regarded as the main point of control for this defence pathway, we describe here an additional mechanism by which the plant can regulate genes of this pathway.

Covalent protein modification by Small Ubiquitin-like Modifier (SUMO) is an important mechanism of signaling regulation. Here we show that the Arabidopsis SUMO protease OTS1 operates as a negative regulator of SA signaling. In comparison to wild-type plants, *ots1* mutants had greatly increased resistance to virulent biotrophic *Pseudomonas syringae* strains. *ots1* could be rescued by expression of a wild-type copy of *OTS1* but not a SUMO protease-deficient version of *OTS1*. Surprisingly, increased pathogen resistance was not found in *ots2* mutants (OTS1 closest homologue), nor did *ots2* enhance the *ots1* mutation. This suggests a specific OTS1-to-target interaction with respect to pathogen resistance.

ots1 mutants showed highly elevated expression of SA marker genes (*PR-1, PR-2* and *PR-5*) even in the absence of treatment. Subsequent analysis revealed that the alterations in expression were not due to changes in the SA level; indicating that perhaps the *ots1* mutation may confer increased resistance independently of SA. To test this, we produced *NahG ots1* double mutants where the *NahG* transgene product degrades all SA, thus effectively producing an *ots1* mutant in which SA does not accumulate. As a result of the cross with *NahG, ots1* was no longer able to confer resistance to *P. syringae*, and had greatly reduced expression of the SA marker genes (*PR-1, PR-2* and *PR-5*). Taken together this evidence suggests that the SUMO protease OTS1 is a novel negative regulator of the SA defence pathway which desensitizes SA-dependent responses.

P344 An E3 Ubiquitin ligase triplet negatively regulates PAMP-triggered immunity in Arabidopsis

Marco Trujillo1, Kazuya Ichimura2, Catarina Casais3, Ken Shirasu2

1Julius-von-Sachs Institute for Biosciences - University of Würzburg, Würzburg, Germany, 2RIKEN Plant Science Center, Yokohama, Japan, 3Sainsbury Laboratory, Norwich, UK

We identified a triplet of closely related plant u-box type E3 ubiquitin ligases (PUBs) that regulate PAMP-triggered signalling. Perception of PAMPs is mediated by plasma membrane localized receptors, such as FLS2, which binds to flagellin. Its activation results in a variety of reactions that include the production of reactive oxygen species (ROS), the activation of mitogen-activated protein kinases (MAPK) and the induction of defence related genes.

Treatment of the *pub22/pub23/pub24* triple mutant with *flg22*, the active epitope of flagellin, induced an increased and prolonged oxidative burst, the selective prolongation of the MPK3 activity andP2 a faster and enhanced transcriptional response. Importantly, the enhanced oxidative burst could be observed after induction with several PAMPs, indicating that the PUB triplet modulates a process common to signalling mediated by several pattern recognition receptors (PRRs). We also showed that PUB22, PUB23 and PUB24 display ubiquitin ligase activity *in vitro*. Together, data shows that the PUB triplet acts in concert to negatively regulate signalling triggered by PAMPs.

Recent results give clues as to the possible cellular mechanisms that might be regulated by the PUB triplet. In a yeast-two-hybrid screen using PUB22 as bait, we isolated putative ubiquitination targets. Several of the PUB22 target candidates are involved in intracellular vesicular traffic. One example is a subunit of the vesicle tethering complex Exocyst. Further analysis to confirm the putative ubiquitination targets of PUB22 and their function during PAMP-triggered immunity are underway.

P345 Microbe-associated molecular pattern-induced Ca^{2+} signalling in *Arabidopsis thaliana*

Stefanie Ranf, Justin Lee, Dierk Scheel

Leibniz Inst. of Plant Biochemistry, Dept. Stress & Dev. Biol., Weinberg 3, 06120 Halle

During attempted infection pathogens betray themselves through conserved molecular structures, so called MAMPs (Microbe-Associated Molecular Patterns). These are recognized by host receptors and activate intracellular signalling cascades leading to innate immune responses. One of the earliest signalling events is a rapid change in the cytosolic Ca^{2+} concentration [Ca^{2+}], which is a prerequisite for downstream responses.

The bacterial MAMPs, *flg22* (a 22-aa peptide of bacterial flagellin) and *elf18* (a 18-aa peptide of bacterial elongation factor EF-Tu), induce defence responses in Arabidopsis,

such as an oxidative burst, MAPK activation and defence gene expression. Both peptides provoke a prolonged [Ca^{2+}], increase in Arabidopsis with a similar lag time, amplitude, duration and shape in a dose-dependent manner. Inhibition of [Ca^{2+}], increase with LaCl3 or BAPTA almost completely abolishes downstream oxidative burst and MAPK activation. Additionally, the non-proteinaceous elicitors peptidoglycan, lipopolysaccharide and chitin oligomer also induce a [Ca^{2+}], increase in Arabidopsis.

Despite the crucial role of [Ca^{2+}], changes in MAMP-signalling, little is known about the identity of the channels/pumps and the diverse Ca^{2+} stores involved and their regulation. Therefore, we tested mutants of known signalling components for their involvement in Ca^{2+} signalling, e.g. *fls2*, *efr, bak1, rbohD* and *mpk3/6*. Furthermore, using EMS-treated apoaequorin-expressing Arabidopsis seedlings,[1] we screened for mutants with altered [Ca^{2+}], increase in response to *flg22* (CHANGED CALCIUM ELEVATION, *cce*). This led to the isolation of new alleles of *fls2/bak1* and other mutants with partially reduced or enhanced [Ca^{2+}], changes after elicitation with several MAMPs. Some cce mutants also show a reduced *flg22*-induced growth inhibition compared to the wild-type. Available mutants will be further characterized using various biotic/abiotic stimuli. Eventually, the *cce* mutants will be useful for elucidating the role of Ca^{2+} in early signalling events in plant/microbe interactions.

Knight *et al* (1991) Nature 352, 524-526

P346 Genetic analysis of *Burkholderia* elicitor responses in Arabidopsis

Natalia Dinischiotu, Bart Feys

Imperial College London, UK

Primarily known for their detrimental characteristics, Burkholderia species have been the focus of intense research over the past decade, mainly due to the discovery that certain Burkholderia strains are important opportunistic pathogens of Cystic Fibrosis patients. Although a diverse array of potential virulence factors have been investigated, their role in pathogenesis remains unclear. Previous studies have emphasized various analogies in innate immunity against pathogens in plant, invertebrate and mammalian hosts. Despite Burkholderia species being able to infect both plants and animals, so far only *in vivo* animal models have been used for research. By comparison, plant models are an attractive option and experiments using *Medicago sativa* have shown potential for this approach with Burkholderia. The aim of this project is to investigate phenotypic responses of Arabidopsis to Burkholderia. Studies performed so far suggest that the phenotypic reactions seen after Burkholderia reflect a strong elicitor recognition event which, is dependent on the SA-pathway. Furthermore, QTL analysis conducted using both a RIL and a NIL population, has revealed that a single dominant gene on chromosome IV controls the chlorotic elicitor reaction displayed by Arabidopsis.

P347 Arabidopsis class II TGA transcription factors are essential for the jasmonic acid/ethylene-induced *PDF1.2* expression

Mark Zander1, Sylvain La Camera2, Olivier Lamotte3, Jean-Pierre Métraux2, Christiane Gatz1

1Albrecht-von-Haller Institute for Plant Sciences, Georg-August-Universität Göttingen, Germany, 2Université de Fribourg, Departement de Biologie, Switzerland, 3UMR CNRS 5184, INRA 1088, Plante-Microbe-Environnement, Université de Bourgogne, France

Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) are crucial signaling molecules orchestrating plant defense responses against biotrophic and necrotrophic pathogens, respectively. In *Arabidopsis thaliana*, a strong suppressive effect of SA on JA-mediated responses is observed. Activation of SA-dependent defense genes requires one of the three redundant class II bZIP transcription factors TGA2, TGA5 or TGA6 which interact with the ankyrin repeat protein NPR1 to establish a state of immunity called systemic acquired resistance.

Here we show that class II TGA factors are also required for the induction of the JA/ET-responsive genes *PDF1.2* and *bCHI*. Moreover, the *tga256* triple mutant was more susceptible to the necrotrophic fungus *Botrytis cinerea*. ET/JA-induced *PDF1.2* expression was restored in the *tga256* triple mutant background by mutating the JIN1 allele, indicating that TGA factors are required to counteract the strong repressive effect of transcription factor AtMYC2/JIN1. Whereas JA/ET-induced *PDF1.2* expression in WT plants is antagonized by SA, it is SA-insensitive in the *tga256 jin1* quadruple mutant. These results reveal that the antagonism between class II TGA factors and AtMYC2 is required for the SA-mediated suppression of JA/ET-induced *PDF1.2* promoter activity. It is concluded that the deployment of class II TGA factors as essential activators of two competing defense programs creates a central control point which prioritizes SA responses upon simultaneous attack by different pathogens.

P348 Posttranslational modifications of SGT1 and their consequences for plant disease resistance

Sabina Zuzga, Rafal Hoser, Jacek Hennig, Magdalena Krzymowska

Institute of Biochemistry and Biophysics PAS, Pawinskiego 5a, 02-106 Warsaw, Poland

The ubiquitin ligase-associated co-chaperone protein SGT1 has been shown to be required for plant immune responses, cell death and hormone signaling. It forms an integral component of a multi-protein network that includes RACK1, Rac1, RAR1, RBOH, HSP90 and HSP70, as well as the rice MAPK, OsMAPK6. OsMAPK6 is an ortholog of SIPK in tobacco (NtSIPK) and of AtMPK6 in Arabidopsis, and these MPK6 orthologs are known to help control the innate immunity response in plants.

Despite the knowledge that both SGT1 and MPK6 orthologs are required for R-gene mediated resistance, the functional relationship between these two signaling

molecules has remained unexplored. Our studies reveal that SGT1 undergoes specific phosphorylation in the canonical MAPK target-SP-motif. The same C-terminal motif is present in most known plant SGT1 proteins, and in the *Saccharomyces cerevisiae* Sgt1, but is absent from the metazoan orthologs. The phosphorylation site lies within a highly conserved domain of SGT1 referred to as SGS (SGT1-specific), the disruption of which leads to loss of many SGT1 activities in plant stress signaling. As the mutant AtSGT1b protein lacks its MAPK phosphorylation site we speculate that the observed phenotype is at least partially attributable to improper posttranslational modifications. Phosphorylation can affect various properties of proteins: subcellular localization, ability to form complexes, stability, conformation, binding of divalent cations.

These aspects are currently under investigation. We will explore which of them are regulated by modification of SGT1 and may be related to SGT1 function in plant defense signaling.

P349 SNO mediated regulation of *AtGSNOR1* is dependent on TTSS and W-box motifs

Noor Saidi, Gary Loake

University of Edinburgh, Edinburgh, Scotland, UK

Nitric oxide (NO) and S-nitrosothiols (SNOs) are widespread signalling molecules that regulate immunity in animals and plants (Wendehenne *et al*, 2001). Previously, we have reported that *Arabidopsis thaliana S-nitrosoglutathione reductase, (AtGSNOR1)* modulates the extent of total cellular SNO formation, which subsequently regulates multiple modes of plant disease resistance (Feechan *et al*, 2005). Loss-of-function mutations in *AtGSNOR1*, leading to increased SNO levels, have recently been shown to result in S-nitrosylation of the key defence regulators NPR1 and AtSABP3, blunting their activity and subsequently leading to increased pathogen susceptibility (Tada *et al*, 2008; Wang *et al*, 2009). Thus, *AtGSNOR1* function would provide a good potential target for pathogen effector proteins. Here, we show that *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pst*DC3000) suppresses *AtGSNOR1* transcript accumulation during early stages of infection and furthermore that this suppression is type III secretion system (TTSS)-dependent. In conjunction, *Pst*DC3000 also increases cellular SNO levels in a TTSS-dependent manner. Deletion analysis of the *AtGSNOR1* promoter identified a region necessary for suppression of *AtGSNOR1* transcript accumulation following *Pst*DC3000 infection. This region includes 3 putative W-box motifs, indicating that transcription factors of the WRKY family may play a key role in mediating this response. Interestingly, *AtGSNOR1* transcript accumulation is further suppressed during early stages of infection by *Pst*DC3000 in *wrky70* mutant. Future work will focus on identifying the specific effector protein(s) that target *AtGSNOR1* and an in-depth study of the interaction between *AtWRKY70* and the *AtGSNOR1* promoter.

P350 Is there a link between actin cytoskeleton and PIP2-dependent phospholipase D in early defense responses during biotic stress

Zuzana Novotna1, Jindriska Matouskova1, Katerina Schwarzerova2, Lenka Burketova3, Olga Valentova1

1Institute of Chemical Technology, Department of Biochemistry and Microbiology, Prague, Czech Republic, 2Charles University Faculty of Science, Department of Plant Physiology, Prague, Czech Republic., 3Institute of Experimental Botany, Academy of Sciences of Czech Republic, Prague, Czech Republic

Plants are continuously resisting damage by potential pathogens using various defense mechanisms for example production of defense-specific chemical messengers such as salicylic acid (SA). Also the cytoskeleton can adaptively reorganize itself in response to a variety of external stimuli, including biotic stresses. In addition, the physical state of the actin cytoskeleleton is a critical determinant of phospholipase D (PLD) activity regulation. Although previous reports imply a role for the actin cytoskeleton and PLD activity in early defense responses, molecular mechanisms are still unknown.

In this study we investigated the changes in the organization of the actin cytoskeleton during salicylic acid treatment of Arabidopsis plants and T-DNA insertion mutants of particular PLD isoforms. To image actin cytoskeleton *in vivo*, transgenic seedlings stably expressing green-fluorescent protein (GFP) fusions with actin binding domain (ABD) of fimbrin (GFP-FABD2) were used. Seedlings were imaged by confocal laser scanning microscopy. Plants were treated with different concentrations of salicylic acid and extensive depolymerization of actin filaments in the hypocotyls and roots of *Arabidopsis thaliana* was induced within minutes of SA treatment. Further, immunofluorescence microscopy of roots and hypocotyls of *Arabidopsis thaliana* was used for investigation of cytoskeleton reorganization in T-DNA insertion mutants of PLD isoforms during SA treatment. We propose that actin cytoskeleton and PIP2-dependent phospholipase D are required for early defense responses to the infection.

This work was supported by the Grant of the Ministry of Education, Youth and Sports of the Czech Republic, project no. LC06034.

P351 Interaction between *Arabidopsis thaliana* and its associated plant growth promoting rhizobacteria

Silke Karojet, Sabine Kunz, Waltraud X Schulze, Joost T van Dongen

Max Planck Institute of Molecular Plant Physiology, Am Muehlenberg1, 14476 Potsdam-Golm, Germany

Plants are generally colonised by various bacteria. But only some of those bacteria are pathogens, whereas others are known to improve growth and health of the host plant. We isolated bacteria that are naturally colonising Arabidopsis roots to establish a test-system for investigating this beneficial plant-bacteria interaction. Several of those bacteria enhanced plant growth after inoculation of sterile seeds. In a proteome profiling approach we screened for plant proteins that are phosphorylated after

bacterial inoculation to find candidates that are involved in the recognition of beneficial bacteria and in the related signalling pathways of the plant. Root associated bacteria were isolated from *Arabidopsis thaliana* ecotype Gol-1 growing in the Berlin-Potsdam area (Germany). Among the bacterial strains we identified several *Pseudomonas* species and a micro-aerophilic *Microbacterium* species that was previously unknown. Inoculation of *A. thaliana* Gol-1 or Col-0 with *Pseudomonas sp*. strain G62 or *Microbacterium sp*. strain G72 enhanced plant growth and stimulated development under various growth conditions.

In a second approach, we investigated how plants recognise beneficial bacteria. Although plants developed a wide array of defence strategies to protect themselves against colonisation and infection by pathogenic micro-organisms, beneficial bacteria are able to colonise the plant. To determine how Arabidopsis recognises and selects for beneficial bacteria, we performed a proteome profiling analysis using LC-MS/MS on liquid Arabidopsis cell cultures. We focussed our analysis on changes in the phosphorylation of membrane proteins after bacterial inoculation. For optimal quantitative comparison of the protein composition, Arabidopsis cells were grown either on ^{14}N- or ^{15}N-isotope labelled nitrate as single nitrogen source. Inoculation with our plant-growth-promoting bacteria or a pathogenic Pseudomonas strain revealed various changes in the phosphorylation of membrane proteins, which are likely involved in the bacterial recognition process.

P352 Ontogenic resistance to powdery mildew is mediated via reduced penetration

Angela Feechan, Hanna Pak, Ian Dry
CSIRO Plant Industry, Adelaide (SA), Australia

Plant development is an important factor for the establishment of pathogen infection. Age-related or ontogenic resistance has been observed in several plant species to viral, bacterial, oomycete and fungal pathogens (Develey-Rivière and Galiana, 2007). For example, both the berries (Gadoury *et al*, 2003) and leaves (Feechan, unpublished) of winegrapes (*Vitis vinifera*) exhibit ontogenic resistance to the host powdery mildew species *Erysiphe necator*. Powdery mildew is an obligate biotrophic pathogen which uses a specialised appresorium structure to generate pressure and enter the plant cell via a penetration peg, where it obtains nutrients using a haustorium.

Arabidopsis is a host for the powdery mildew species *Golovinomyces cichoracearum*. However wild-type Col-0 plants display ontogenic resistance to *G. cichoracearum*, showing a decrease in penetration rates from 82% ±7 in 2 week old plants to 38% ±17 in 6 week old plants. Leaves of different developmental stages were also found to differ in ontogenic penetration resistance. Early rosette leaves from 6 week old plants were found to display lower penetration rates (38%±17) compared to leaves formed later in development (62%±2).

The Arabidopsis mutant, *pen1-1* was previously isolated in a screen to identify mutants which allow penetration and haustoria formation by non-adapted powdery mildews (Collins *et al*, 2003). More recently it has also been implicated in penetration resistance to the host mildew *G. cichoracearum* (Zhang *et al*, 2006). We have confirmed this observation and have preliminary evidence to suggest that ontogenic penetration resistance to G. cichoracearum may be partially dependent on PEN1.

Collins *et al* Nature 425, 973–977 (2003).
Develey-Rivière and Galiana. New Phytologist 175, 405-416 (2007).
Gadoury *et al* Phytopathology 93, 547-555 (2003).
Zhang *et al* Plant Journal 49, 302-312 (2007).

P353 The peroxidase-dependent oxidative burst and its role in plant defence in Arabidopsis

Arsalan Daudi, Jose O'Brien, G Paul Bolwell
Royal Holloway, University of London, Egham, Surrey, UK

Plants being sessile organisms have evolved a series of defence mechanisms to deal with very diverse biotic and abiotic threats. In order to defend themselves, plants mount a defence response to limit pathogen growth and consequently destroy it. There is a need to dissect the various contributions of these responses to individual stresses. One of the first responses is the rapid and localized production of reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), referred to as the oxidative burst. Previously, it was demonstrated that an Arabidopsis transgenic line transformed with anti-sense cDNA of a french bean class III peroxidase shows an impaired oxidative burst and an increased susceptibility to fungal and bacterial pathogens. In order to continue characterizing the peroxidase-dependent oxidative burst we analysed this mutant together with single mutants of the two main peroxidases involved (At3g49120 and At3g49110), the double mutant and an over-expression line of At3g49120. In addition to the peroxidase mutants several additional mutants identified by microarray analysis of the original antisense mutant were analysed as well. A combination of different approaches involving physiological screening, genomics, proteomics and metabolomics technologies have been applied to analyse these responses. In the present work we show that the lack of H_2O_2 production in the initial state of pathogen infection leads to an impaired defence response, as shown with decreased callose deposition. In addition, we demonstrate that this response can be completely recovered by adding exogenous H_2O_2. This data gives evidence of the crucial role of hydrogen peroxide and peroxidases in plant defence and the subsequent activation of plant defence responses such as callose deposition.

P354 Dual role of the ER-resident glucosidase in MAMP-triggered immunity and abiotic stress responses

Xunli Lu, Nico Tintor, Silke Robatzek, Paul Schulze-Lefert, Yusuke Saijo
Max-Planck Institute for Plant Breeding Research, Dept. of Plant Microbe Interactions, Cologne, Germany

Plants detect microbe associated molecular patterns (MAMPs), thereby triggering innate immune responses to restrict the invasion and growth of microbes. The

Arabidopsis Leucine-rich repeat receptor-like kinases EFR and FLS2 respectively recognize bacterial MAMPs EF-Tu and flagellin (and their bioactive epitopes elf18 and flg22). The molecular basis for MAMP-triggered immunity is largely unknown. We established genetic screens for MAMP-insensitive mutants based on an interesting phenomenon that MAMPs block sucrose-induced flavonoid accumulation in young Arabidopsis seedlings. We isolated *priority in sweet life (psl)* mutants that are impaired in *elf18*-induced, but not *flg22*-induced, signalling. Consistent with this, *psl4* and *psl5* mutant plants show hypersusceptibility to the virulent bacterium *Pseudomonas syringae* pv. *tomato* DC3000. The steady-state levels of EFR, but not FLS2, are greatly reduced in *psl4* mutants. We identified that *PSL4* and *PSL5* encode Glucosidase IIb and a subunits respectively. Glucosidase is conserved in eukaryotes and acts in the endoplasmic reticulum (ER). This enzyme regulates the N-glycosylation of membrane-localized proteins that are subject to folding and maturation by the calnexin/calreticulin cycle, of which two other components have also been defined as PSL1 and PSL2. Our data suggest that EFR is a client of this ER-resident protein quality control system. We further observed that *psl4* and *psl5*, but not *efr*, mutant plants show a swollen root phenotype under high sucrose conditions, indicating the existence of another client protein apart from EFR that maintains root integrity upon the abiotic stress. Our findings identify the ER-resident protein quality control system as a convergence point of biotic and abiotic stress responses in plants.

P355 Unravelling the oxylipin signalling pathways that modulate plant stress responses

Michael Bartsch, Carmen Castresana

Centro Nacional de Biotecnología, Dep. of Plant Molecular Genetics, Lab. 315, C/ Darwin 3. 28049 Madrid

Plants recognize invading pathogens and trigger an array of signalling events eventually leading to a resistance response that prevents or limits pathogen growth *in planta*. One signalling event upon pathogen infection is the formation of lipid derived oxylipins. In animal physiology a broad signalling role of fatty acid derived molecules (eicosanoid family of lipid signals) has been well established. Increasing evidence is emerging that plant oxylipins have a similar complex and vital biological role as signalling molecules contributing to resistance. In an *in vitro* assay, application of the oxylipin 9-hydroxyoctadecatrienoic acid (9-HOT) triggered potent effects in Arabidopsis wild-type seedlings like a root waving phenotype and the induction of hallmarks of plant defence. From a forward genetic screen approximately 60 mutants were identified that were non responsive to the oxylipin 9-HOT (*noxy* mutants). A subset of these mutants have been tested in pathogen assays and revealed altered defence responses (enhanced or decreased resistance). The task will now be to identify the mutated genes and to perform their functional characterisations. Based on the phenotypical analysis of the *noxy* mutants we expect to identify genes that are of central importance for the perception and transduction of oxylipin signals in plant immunity and abiotic stress resistance.

Natural Variation

P356 The QTLs for floral and leaf morphology and natural variation in *Arabidopsis thaliana*

Umesh Reddy1,2,3, Mohammed Rahman1, Padma Nimmakayala1

1Department of Biology, 2West Virginia State University, 3Institute, WV 25112, USA

One hundred recombinant inbred lines of the cross Col x Ler were evaluated in the multiple environments for various floral (length and width of petal, sepal, pistil and anther as well as rosette and cauline leaf characters (LAI, length and width), and identified several QTL locations throughout the genome. We noted many QTLs that are linked to various traits from the non-coding part of the genome, which do not possess known floral/leaf genes. Thirty ecotypes belonging to the diverse geographical regions were grown in replications for collecting the data pertaining to natural variation among the observed QTLs. We subjected the data to association mapping and obtained interesting results pertaining to causative SNPs underlying the natural variation. Results pertaining to association mapping will be discussed.

P357 Natural variation in *Arabidopsis thaliana* as a tool for identifying differentially expressed genes involved in the elicitation process of pharmacologically active plant secondary metabolites

Anna Ihnatowicz1, Joanna Siwinska1, Matthieu Reymond2, Ewa Lojkowska1

1Intercollegiate Faculty of Biotechnology UG-MUG, Gdansk, Poland, 2Max Planck Institute for Plant Breeding Research, Cologne, Germany

Plants produce a wide variety of secondary metabolites that have a range of important functions and biological activities valuable for both plants and humans. They are produced in plants as a defence mechanism against pathogen attack and other stress events, and serve as key signalling compounds in mutualistic interactions and plant development. Numerous plant secondary metabolites have significant biological, pharmacological and therapeutic activities in humans, and have found medical application in the treatment of various diseases. Even though plants are a good biotechnological source of biologically active compounds, the commercial production of secondary metabolites using plant culture is normally limited by their low yield. Therefore better understanding of the elicitation process, which is an extensively used tool for enhancing secondary metabolite yields, is of great importance. In spite of the fact that all medicinal plants currently studied are non-model organisms, *Arabidopsis thaliana* with its extensive genetic natural variation, and its tools of molecular biology, biochemistry, and functional genomics provides an excellent model to study plant secondary metabolism and elicitation process. The aim of this

work is to identify differentially expressed genes that determinate the phenotypic variation in secondary metabolite accumulation between Arabidopsis accessions treated with various elicitors. Sets of Arabidopsis natural accessions originating from a wide range of habitats were grown together in control conditions and were subsequently treated with different biotic and abiotic elicitors. Harvested plants are being used for the parallel analysis of transcript profiling using a fluorescent differential display technique and metabolic profiling of secondary metabolites. The long-term aim of the project is to expand molecular understanding of secondary metabolite biosynthesis at an ecological level in order to get insight into elicitation process.

P358 Combining Genome Wide Association mapping and QTL mapping in *Arabidopsis thaliana* for detecting genes underlying flowering time natural variation measured in ecologically realistic conditions

Benjamin Brachi1, Nathalie Faure1, Matt Horton2, Joy Bergelson2, JoÎl Cuguen1, Fabrice Roux1

1Laboratoire de Genetique et Evolution des populations vegetales, Universite de Lille1, France, 2Department of Ecology & Evolution, University of Chicago, IL USA

In plants, flowering time determines the transition from the vegetative phase to the reproductive phase. During this second phase, the plant reallocates resources it has accumulated during the vegetative growth phase in order to produce seeds. In annual plants, all resources should be reallocated to offspring production since there need not be investment in long-term survival. Flowering date has therefore been extensively studied, especially in the model annual plant species *Arabidopsis thaliana*. For this particular species, at least 100 genes have been shown to be implicated in the determinism of flowering time. While the different metabolic pathways leading to flowering have been largely unravelled, many questions remain, especially from an evolutionary point of view.

The identification of flowering time genes and the pathways leading to early or late flowering has been based on laboratory mutants and phenotypes obtained in greenhouse conditions. But which ones actually underlie the natural variation for flowering date observed in nature?

In order two answer this question, we combined Genome-Wide Association mapping and classical QTL analyses to detect flowering time genes. The GWA-mapping is based on 197 natural accessions genotyped for more than 240,000 SNP markers. The QTL analyses are performed using 13 RIL families including ~ 300 strains, each genotyped for an average of 85 markers. This combination allows us to benefit from the fine mapping possible with GWA while simultaneously controlling for false positives with the RIL families. Overall, more than 18,000 plants where phenotyped in a single, common garden experiment in northern France. This allows us to detect which flowering time genes underlie the observed natural variation in *Arabidopsis thaliana*.

P359 Natural variation and quantitative genetics in Arabidopsis highlights a tight relationship between root and shoot growth that loosen under water deficit

Marie Bouteillé1, Olivier Loudet2, Detlef Weigel3, Bertrand Muler1

1INRA, LEPSE, Montpellier, France, 2INRA, Plant Genetics, Versailles, France, 3Max-Planck-Institute for Developmental Biology, Tubingen, Germany

Growth of leaves and roots are two intimately related processes, in particular through developmental or nutritional clues and their balance is under environmental control. Increases in root/shoot ratio under water or mineral deficit could contribute to the adaptation to poor resource environments. We therefore explored the possibility that this balance and its response to soil water deficit is under genetic control in Arabidopsis.

First, the range of variation of this balance was explored in a set of 20 'Perlegen' accessions, using the Phenopsis platform to impose rigorously controlled soil water deficit (Granier *et al* 2006). Contrasted behaviours of the accessions could be distinguished, based on their degree of shoot growth decrease and root growth maintenance under water deficit. The Bay-0 x Sha RILs population was chosen for QTL analysis from the contrasted response of the parents.

In experiments performed in hydroponics or in soil under well watered conditions, most growth QTLs identified were involved in the control of both root and shoot growth. Different variables were thus derived to identify the genetic basis of root or shoot growth (residual of the root shoot relationship, coordinates along PCA axis). A consensus region at the top of chr 3 affecting both primary root length and biomass partitioning was identified and further confirmed using Heterologous Inbred Families.

In soil under water deficit, regions controlling root and shoot growth were more loosely related and residual/PCA analysis allowed the identification of regions controlling root or shoot growth or their response to soil water deficit independently. Together, these results show that the strength of the root – shoot relationship is translated at the genetic level, that numerical tools are available to identify regions specifically controlling root growth and that water deficit tend to loosen this relationship suggesting that root growth is more sink limited under water deficit than in normal conditions.

P360 How does natural variation play a role in adaptation?

Poay Ngin Lim, Andrew Hudson

Institute of Molecular Plant Sciences, University of Edinburgh, Edinburgh, UK

Numerous studies on natural variation between worldwide ecotypes of Arabidopsis have been reported. However, it is unclear how natural variation plays a role in adaptation in local natural populations, where individuals are more similar to each other than worldwide

accessions. We have examined natural variation in populations of Arabidopsis collected around Edinburgh and found a high level of genetically determined variation in a number of traits, including growth rate and flowering time. Crossing fast and slow growing plants and carrying out bulk segregant analysis on hybrids suggests at least two growth associated Quantitative Trait Loci are involved in this local variation. We also examined vegetative growth in these wild Arabidopsis families under varying natural conditions, including different seasons and elevations. Our studies suggest that some genotypes perform better as winter annuals and some as summer annuals. Responses to key seasonal environmental factors such as temperature, day length and light intensity may be involved in adaptation to a winter or summer annual lifestyle, and provide important clues to explain how the adaptive trait might have evolved.

P361 Polymorphisms in *BRX* are associated with natural variation in Arabidopsis primary root growth

Julien Beuchat1, Chikako Shindo1,2, Stephanie Plantegenet1, Christian S Hardtke1

1Department of Plant Molecular Biology (DBMV), University of Lausanne, Switzerland, 2Present adress: Bayer Crop Science, Gent, Belgium

Root system architecture is marked by a high plasticity and is shaped by both intrinsic developmental pathways and environmental responses. Recent studies that aimed at understanding the mechanisms underlying this morphological variation have revealed new genes that are important for the regulation of root growth. For instance, *BREVIS RADIX (BRX)* was isolated through a naturally occurring allele in the *Arabidopsis thaliana* accession Uk-1 and was shown to regulate cell proliferation and elongation in the root. *BRX* is the founding member of a highly-conserved plant-specific gene family. Since BRX plays an important role in root growth, we wanted to determine whether polymorphisms in *BRX* could contribute to variation in primary root length among *A. thaliana* accessions by a combined haplotyping phenotyping approach. On the one hand, the haplotype data revealed that *BRX* and its homolog *BRX-like* 1 are highly conserved across accessions. On the other hand, we found a 7 amino acid deletion in BRX in two accessions, Lc-0 and Lov-5, which displayed above average primary root length. A recombinant inbred line population between Lc-0 and Eil-0 allowed us to address whether this deletion is associated with natural variation in root growth. Indeed, plants that carried the Lc-0 allele showed slightly but significantly enhanced primary root growth, suggesting that the 7 amino acid polymorphism is functionally relevant. This may indicate that variation of BRX activity among accessions could be due to differences in the amino acid sequence rather than changes in regulatory mechanisms.

Details on direct tests of allele activity in a standardized background will be reported.

P362 Robust associations in *Arabidopsis thaliana* for GWA mapping of quantitative resistance to the pathogen *Pseudomonas viridiflava*

Fabrice Roux1, Matt Horton2, Joy Bergelson2

1Laboratoire de Genetique et Evolution des Populations Vegetales, UMR CNRS 8016, Universite des Sciences et Technologies de Lille1, F-59655 Villeneuve dascq cedex France, 2Department of Ecology & Evolution, University of Chicago, Chicago, IL 60637, USA

Because pathogen species are a threat for crops and natural plant populations, a main challenge is to identify the genetic basis underlying variation in resistance in plant species. In *Arabidopsis thaliana*, Genome-Wide Association (GWA) mapping has been shown to be successful for identifying pathogen resistance genes involved in the qualitative response (hypersensitive response, HR) to the strain *Pseudomonas syringae:* Pst DC3000 (Aranzana *et al* 2005). We applied this method to identify the genetic basis of quantitative resistance to *Pseudomonas viridiflava*, pathogen species affecting plant yield throughout the world.

Combining phenotyping (quantitative resistance to different *P. viridiflava* strains) and genotyping (213kSNPs) of a worldwide collection of 192 *A. thaliana* accessions, and a mixed-model approach for reducing the false-positive rate while maintaining statistical power, we identified *A. thaliana* genomic regions involved in the quantitative resistance to *P. viridiflava.* A study of the genomic regions around the significant SNPs led us to class candidate genes in 3 categories: i) disease resistance genes (TIR genes), ii) genes described in the literature as being involved in the response to pathogens, and iii) genes with sequence homologies to resistance genes in other species.

Aranzana MJ, Kim S, Zhao K, Bakker E, Horton M, Jakob K et al (2005). Genome-Wide Association mapping in Arabidopsis identifies previously known flowering time and pathogen resistance genes. PLoS Genetics 1(5): 0531-0539.

P363 Gene transposition causing natural variation for growth in *A. thaliana*

Daniela Vlad, Olivier Loudet

INRA, SGAP UR254, F-7802 Versailles, France

Genetic analysis of natural variation in ecotypes can ease the discovery of new genes and represents a resource that can be exploited to study the genetic regulation of plant growth and response to environmental factors.

We studied natural variation for growth related traits using an *A. thaliana* RIL population obtained from a cross between Bur-0 (Burren, Ireland) and Columbia (Poland). We observed that RIL212 was segregating for a major growth QTL (Quantitative Trait Locus) located at the bottom of chromosome 4, named SG3 *(Shoot Growth-3)*. Despite its great phenotypic consequences *SG3* was not visible in the RIL population due to an epistatic interaction and because it was most likely counter-selected.

Data from the analysis of several independent HIFs segregating for *SG3* showed that the QTL is completely interacting with another locus at the top of chromosome 4, *SG3i (Shoot Growth-interactor)*. The phenotypic effect of *SG3* in an HIF is conditioned by the presence of the Col allele at *SG3i*.

Fine mapping revealed the gene responsible for the phenotype, a stromal oxidoreductase affecting photosynthetic electron transfer rate, which has severe consequences on pigmentation and growth rate.

We found that *SG3* and *SG3i* represent a duplicate gene pair in Bur-0, while only one copy is present in Col at *SG3*. One polymorphism (indel) in the Bur-0 *SG3* copy changes the reading frame resulting in a much shorter protein sequence. Overall, our findings support the hypothesis that the observed phenotypic variation is due to the transposition of the functional copy in the Bur-0 parent.

To get a snapshot into the evolution of this duplicate gene pair at species level, 48 distinct Arabidopsis accessions are being analysed. Preliminary results show the retention of the two copies in more than 90% of the genotypes. Considered to be genetically unstable, like any redundant paralog pair, the *SG3/SG3i* couple in most probably in a transition stage towards redundancy loss.

P364 Mapping of a qualitative trait locus responsible for flavonol glycoside accumulation in newly developed Recombinant Inbred Lines of *Arabidopsis thaliana*

Sandra Frank1, Hirofumi Ishihara1, Rhonda C Meyer2, Margarete Mueller2, Ralf Stracke1, Thomas Altmann2, Bernd Weisshaar1

1Bielefeld University, Dept. of Genome Research, Faculty of Biology, Germany, 2IPK Gatersleben, Dept. of Molecular Genetics, Germany

The main features of flavonoid biosynthesis have been elucidated and regional distinctions between species and even accessions became apparent. Although the underlying pathways have been intensively studied during the past decades, the identification of the participating genes and understanding their regulation is still challenging. Aims of this work are the identification and functional analysis of genes involved in phenylpropanoid-metabolism in the model plant *Arabidopsis thaliana* by a genetic mapping approach with newly developed Recombinant Inbred Lines (RILs) derived from reciprocal crosses of the accessions C24-G1 (Golm1) and Nd-G1. The generated RILs comprise 497 F8 individuals in total, that were genotyped with a frame-work marker set of 142 SNP markers evenly distributed along the five chromosomes. Several differences in seedling metabolite content between the parental lines C24-G1 and Nd-G1 were identified by High Performance Thin Layer Chromatography (HPTLC), concerning flavonol-glycoside accumulation, but also compounds of so far unknown structural identity. Analysis of the F1 generation derived from reciprocal crosses of the parental lines revealed, that accumulation of two flavonol-glycosides is based on the dominant C24-G1 allele, which was mapped to a 40 kb comprising region on the lower arm of chromosome 1.

P365 Genome-wide association mapping of freezing tolerance in *Arabidopsis thaliana*

Glenda Willems1, Bjarni J Vilhjalmsson1, Justin Borevitz2, Joy Bergelson2, Maarten Koornneef3, Magnus Nordborg1

1Molecular and Computational Biology, University of Southern California, Los Angeles, USA, 2Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA, 3Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany

The identification of the genetic variants underlying adaptive traits constitutes one of the major challenges for modern biology. This has been highly facilitated through the recent availability of a wide range of "-omics" tools. However, elucidating the genetic mechanisms contributing to adaptation is still difficult in the case of complex traits. The recent advances as well as the decreasing costs in genotyping technologies have made genome-wide association (GWA) studies feasible. Compared to classical linkage analyses, GWA studies provide the possibility to identify single or a few genes, rather than hundreds of genes. Moreover, GWA can be performed on natural populations, and does not involve pedigrees or controlled crosses. Throughout its worldwide geographic distribution *Arabidopsis thaliana* experiences a broad range of climatic conditions and selective pressures, which makes it an ideal study system for the analysis of adaptive traits such as freezing tolerance. With the aim of identifying the genes underlying freezing tolerance, we performed a GWA study on approximately 250 natural Arabidopsis lines, which have been genotyped for approximately 250,000 Single Nucleotide Polymorphisms (SNPs) using a non-parametric rank-based test, and a parametric test that corrects for population structure. A small number of SNPs (less than 10) were associated with freezing tolerance for the non-parametric, and parametric analysis at a significance threshold of 0.05 corrected for multiple testing. Because of the high false negative rate at this threshold, we focus on the 10 most highly ranked SNPs in the parametric, and non-parametric analysis for further analysis. A total of 41 genes were found to be at a distance of 1kb or less of these SNPs. The role in freezing tolerance of a subset of these genes will be validated using diverse approaches, ranging from fine-mapping to cloning.

P366 Natural variation in temperature compensation in *Arabidopsis thaliana*

Jelena Kusakina, James Hartwell, Anthony Hall

University of Liverpool, UK

Temperature compensation is a characteristic that allows circadian oscillators to maintain a robust and accurate rhythm over a broad range of temperatures. Arabidopsis accessions have previously been shown to exhibit considerable variation in temperature compensation response when analyzed by leaf movement (Edwards *et al*, 2005). In this study, we used a luciferase reporter gene (LUC) fused to *CCA1* and *LHY* promoters to monitor circadian rhythmicity and changes in temperature compensation between 19 Arabidopsis accessions at 17ºC versus 27ºC. All accessions displayed a decrease in circadian period in response to high temperature, however, the

degree of deviation from the norm and rhythm robustness (RAE) varied depending on the accession. These results suggest that in some accessions the temperature range permissive for rhythmicity is extended, which leads to a better capability to buffer the clock against temperature change. Correlation between circadian parameters and physical performance of accessions will be analyzed to check if maintaining a robust clock is important at high temperature.

P367 Natural variation in *Arabidopsis thaliana* revealed a complex genetic determinism of germination under salt stress

Navot Galpaz, Maarten Koornneef, Mathieu Reymond

Max Plank Institute for Plant Breeding Research, Carl von Linne Weg 10, Cologne, Germany

In this study we harness natural genetic variation in *Arabidopsis thaliana* to detect and clone QTLs (Quantitative Trait Loci) involved in plant response to salt stress. Ninety eight accessions of *Arabidopsis thaliana*, collected in contrasting habitats, were screened for the response to 125 and 175 mM NaCl on agar plates. Among these accessions, a wide range of responses to salt (root and shoot development, germination rate and seedlings survival) were observed. Interestingly, different sets of accessions showed tolerance at the level of root development (125 mM NaCl) and germination and survival rate (175 mM NaCl), suggesting that the responses to different levels of stress are controlled by different genetic determinisms. Shakdara (Sha, originated from Tajikistan), showing the highest rate of seed germination and seedlings survival in 175 mM NaCl plates, was selected for further QTL analysis. Three RILs (Recombinant Inbred Lines) mapping populations, having Sha as a common parent; Sha x Col-0 (Columbia), Sha x Ler (Landsberg erecta) and Sha x Bay-0 (Bayreuth) were grown under 0, 125 and 175 mM NaCl. This allowed us to detect 22 significant QTLs. Four QTLs were detected in both Sha x Col and Sha x Ler mapping populations, (top Chr 1 and 2**,** middle chr 3, and bottom chr 5) affect specifically germination rate in 175 mM NaCl plates. Interestingly, epistatic interactions between these QTLs were detected, as only one specific allelic combination at these four loci leads to abolishment of seed germination. Two approaches are being used to clone the genes underlying the effect of the detected epistatic QTLs : (i) a genetic approach is being pursued where suitable HIF (Heterozygous Inbred Families) and NIL (Nearly Isogenic Lines) have been selected. These lines are used to validate and further fine map and clone the detected QTLs. (ii) the biochemical approach (yeast two hybrid); we are using candidate genes to reveal interacting proteins that control seed germination under severe salt stress.

P368 DNA variation in the *PHERES* genes of Arabidopsis species

Akira Kawabe1,2, Alan Forrest2, Deborah Charlesworth2

1National Institute of Genetics, Mishima, Japan, 2University of Edinburgh, Edinburgh, UK

The *Pheres (PHE)* genes are among the known genes imprinted in endosperm cells of the plant *Arabdiopsis thaliana*. To test whether an evolutionary arms race has driven rapid sequence evolution in this component of the imprinting system, we studied DNA variation in the two *A. thaliana PHE* genes, which are closely linked, and in the homologues in its close relative, *A. lyrata.* In *A. thaliana*, strongly reduced divergence between the *PHE1* and *PHE2* loci in a short region (about 100bp) of the 5' flanking region of the genes suggests gene conversion. The conversion events tend to occur from *PHE1* to *PHE2*, causing high diversity in *PHE2*. In *A. lyrata*, the *PHE1* gene is duplicated, but the sequences cannot be classified into two clear clusters, and three similar sequences were found in a single individual plant; both results again suggest gene conversion between the duplicates. A short 5' flanking region is conserved among all the *A. lyrata PHE1* sequences, suggesting purifying selection. The *A. lyrata PHE2* locus diversity is high in the 5' flanking region, with divergent haplotypes. These results suggest natural selection affect diversity patterns of the 5' flanking region of both *PHE* loci.

P369 Evaluation of natural diversity in *Arabidopsis thaliana* accessions: A tool to identify the genetic basis of nitrogen use efficiency

Sobia Ikram, Fabien Chardon, Francoise Daniel-Vedele, Sylvain Chaillou

INRA, IJPB, Unite de Nutrition Azotee des Plantes, 78026 Versailles, France

Plant growth and development ultimately depends upon environmental features, such as temperature, light intensity, availability of water and essential minerals. Nitrogen serves as a very important component for plant development. Improving plant nitrogen (N) use efficiency or controlling soil N requires a better knowledge of the regulation of plant N metabolism. This could be achieved using *Arabidopsis thaliana* as a model genetic system, and taking advantage of the natural variation available among ecotypes. In our experiment, we looked for natural variation in plant responses to diverse nitrogen nutritions within a core-collection of 24 accessions that was shown to maximize the genetic diversity captured for a given number of individual accessions in Arabidopsis. We imposed different nitrogen environmental conditions, e.g. normal nitrogen supply (N^+), limited nitrogen supply (N^-) and absence of nitrogen as a stress (N stress) and measured different morphological and metabolic traits. Plants frequently experience these conditions in the field and natural environments. Our study revealed the plant adaptation to an imbalance of exogenous nitrogen sources by allocating the biomass to the root system that is involved in acquiring the scarce resources. Secondly we measured the variation in response of different accessions. Our results indicated that some accessions show adaptation in nitrogen limited supply and nitrogen stress condition. On the basis of this adaptation we can distinguish three classes. Accessions belonging to three classes showed interesting phenotypic variation in response to imbalance of nitrogen nutrition,which can be exploited to identify genes and alleles important for this complex trait.

P370 Patterns of shared polymorphism between *Arabidopsis halleri* and *A. lyrata:* The role of demographic and selection processes

Camille Roux, Xavier Vekemans, Vincent Castric
GEPV, Lille, France

The process of speciation leading to two closely related species involves at some point the reproductive isolation of a set of individuals from the rest of the ancestral population. Hence, genetic diversity present in the ancestral population at the splitting time will be initially distributed in each of the two subpopulations, and, assuming the reproductive barrier is complete, will then progressively segregate and diverge in response to processes of genetic drift and selection of numerous factors. Under a purely neutral model without demographic perturbartions, A.Clark (1997) showed that the mean time to retain 5% of shared polymorphism is only 3.8Ne generations (Ne is the mean effective population size of the sister species), 5.3Ne generations to retain 1%. Regions subject to selection will show contrasted departure from these expectations. Around a locus which is subjected to a strong balancing selection, the loss of ancestral polymorphism will operate more slowly than in selectively neutral regions. Inversely, diversifying selection will drive a more rapid loss of shared polymorphism than in neutral regions.

In the case where introgression occurs between the sister species during demographic history, introgressed regions should show highest shared polymorphism than the neutral expectation.

We have the project of generating sequence data for different set of loci from natural populations of *A.halleri.* First a control set of anonymous loci, second a set of loci genetically linked to the self-incompatibility subject to strong balancing selection, and third a set of genomic regions candidates for directional selection associated to adaptation to heavy metals polluted soils in *A.halleri.* The data for the control set of loci is already available. Nucleotide polymorphism at this set of loci will be compared to polymorphism found in *A.lyrata* and the extent of shared polymorphism will be estimated. The data will be used to test alternative hypotheses regarding the history of these sister species. In particular we will estimate the main parameters to describe isolation with migration speciation models, i.e. current and ancestral population sizes, split time, and the rate of gene flow.

Cell Biology

P371 A tripartite SNARE-K^+ channel complex involved in Arabidopsis potassium nutrition

Annegret Honsbein1, Sergei Sokolovski2, Prisca Campanoni3, Rejane Pratelli4, Manuel Paneque5, Ingela Johansson1, Michael R Blatt1

1Laboratory of Plant Physiology and Biophysics, University of Glasgow, UK, 2Div. Electronic Engineering & Physics, University of Dundee, UK, 3Philip Morris Products, Neuchatel, CH, 4Carnegie Institution of Washington, Stanford, USA, 5Facultad de Ciencias Agronomicas

In addition to their roles in vesicle delivery and fusion, a few membrane trafficking (SNARE) proteins interact with ion channels, notably mammalian Syntaxin1A which binds to specific Ca^{2+} and K^+ channels in nerves and neuroendocrine tissues to modulate their gating properties. Such interactions have been thought to be restricted to mammalian tissues in which they serve highly specialized roles to facilitate signaling and its coupling to membrane traffic. We discovered that the SNARE protein SYP121 of the model plant Arabidopsis binds directly and selectively to the regulatory K^+ channel subunit KC1, which assembles with different inward-rectifying Shaker K^+ channels to affect their activities. The Shaker subunits AKT1 and KC1 form heterotetramers that are involved in potassium uptake at the root hair and epidermis. We found that SYP121 promotes gating of the inward-rectifying K^+ channel AKT1 when heterologously co-expressed with KC1, and that the SYP121-KC1 complex is essential *in vivo* for AKT1-associated K^+ current, channel-mediated K^+ uptake at the root epidermis and for growth.

These results demonstrate a role for a SNARE as part of protein complex facilitating plant mineral nutrition and they implicate additional roles for SNARE binding to control the activity of other ion channels through the common KC1 subunit.

P372 Dissecting endomembrane trafficking underlying gravitropic responses in *Arabidopsis thaliana* via chemical genomics

Michelle Brown1, Georgia Drakakaki1, Stephanie Robert2, Anna-Maria Szatmari2, Eugenia Russinova2, Jiri Friml2, Natasha Raikhel1, Glenn Hicks1

1University of California, Riverside, CA, USA, 2Ghent University Technologiepark, Ghent, Belgium

Cell elongation and endomembrane trafficking are components of the root gravitropic response. In order to better understand the relationship between gravitropism and endomembrane trafficking, we employed a chemical genomics strategy to identify endomembrane compartments involved in the gravitropic response.

A high throughput screen exploited the highly active and tip-focused plasma membrane cycling in pollen tubes. In this screen we assayed tens of thousands of small molecules and identified several hundred that inhibited pollen germination due to inhibition of endomembrane trafficking, among other possibilities.

The compounds were assayed for protein mis-localization in Arabidopsis roots in a line expressing a GPF fusion to PINFORMED2 (PIN2), an auxin efflux carrier involved in auxin re-localization during gravitropic responses. PIN2:GFP normally localizes to the plasma membrane and endosome compartments. Thirteen mis-localization phenotypic categories were observed amongst the compounds that disrupted PIN2:GFP localization. These compounds will help define the intersection of endosomal cycling and gravitropic responses.

P373 Functional modules in the Arabidopsis core cell cycle binary protein-protein interaction network

Joanna Boruc1, Hilde Van den Daele1, Jens Hollunder1, Stephane Rombauts1, Evelien Mylle1, Rebecca de Clerq1, Pierre Hilson1, Dirk Inze1, Lieven De Veylder1, Eugenia Russinova1

1VIB Department of Plant Systems Biology, Ghent University, Belgium

As in other eukaryotes, cell division in plants is highly conserved and controlled by cyclin-dependent kinases. The activity of these kinases is predominantly regulated at the post-transcriptional level by their association with regulatory proteins, such as cyclins. Although over the last years our knowledge of the plant cell cycle has considerably increased, little is known on the assembly and regulation of the different complexes. To map protein-protein interactions between core cell cycle proteins of *Arabidopsis thaliana*, a binary protein-protein interactome network was generated with two independent high-throughput interaction assays, yeast two-hybrid and bimolecular fluorescence complementation. Pairwise interactions among 58 core cell cycle proteins were tested, resulting in 348 interactions of which 283 have not been reported before. Integration of the binary interaction results with cell cycle phase-dependent expression information and localization data allowed the construction of a dynamic interaction network. The obtained interaction map constitutes a framework for further in-depth analysis of the cell cycle machinery.

P374 Why tetraploid cells are larger than diploid cells in Arabidopsis? Application of an artificial tetraploidization method to Arabidopsis research

Hirokazu Tsukaya

Graduate School of Science, The University of Tokyo, Bunkyo-ku, Tokyo 113-0033, Japan and National Institute for Basic Biology, Okazaki 444-8585, Japan

We are interested in mechanisms that control leaf shape/size. In the present study, the relationship between ploidy level and leaf-cell size was analyzed in Arabidopsis. Polyploid formation is very common and one of key events for diversification of characters in plants. Polyploid formation is also an exceptional evolutionary process that can be reproduced experimentally. It is widely known that high ploidy level results in larger cell size in animals, fungi, and plants, however, an explanation to this phenomenon remains to be provided.

In Arabidopsis, palisade cells of the tetraploid are twice larger in volume than the diploid. The tetraploid has also a doubled amount of RNA than the diploid. Is this increase in RNA amount the cause of the increase in cell size?

To understand the fundamental relationship between the ploidy level and cell size, we established a protocol of tetraploidization of Arabidopsis strains using colchicine. Then a series of small-celled and/or leaf-shape mutants were tetraploidized and analyzed for their cell size, afterwards. As a result, unexpectedly, we found that the genetic background strongly affects the ratio of size between the original diploid and the induced tetraploid, namely, the ratio varied from 1.2 to 2.9 while it is ca. 1.8 in wild type. Our data clearly denies a naïve hypothesis that says: cell size automatically reflects the amount of RNA that is proportional to the copy number of genomic DNA. Based on our results, some possible mechanisms between ploidy level and cell size control will be discussed.

1 Tsukaya H. (2008) Two mysteries regarding organ-wide size/shape control in a multicellular organ: the leaf. PLoS Biology 6, 1373-1376.
2 Breuer *et al* (2007) Arabidopsis BIN4, a novel component of the plant DNA topoisomerase VI complex, promotes organ growth by endoreduplication. Plant Cell 19, 3655-3668

P375 Functional analysis of fused-kinase signalling in gametophytic cytokinesis

Trudie Allen1, Sung-Aeong Oh2, Anna Sidorova1, David Twell1

1Biology, University of Leicester, UK, 2Kyungpook National University, South Korea

The partitioning of the cytoplasm following mitosis occurs via distinct mechanisms in plant and animal cells. In contrast to animal cells that divide by assembly of an actomyosin contractile ring, plant cells build an expanding cell plate with the aid of the plant-specific cytokinetic phragmoplast. Phragmoplast expansion leading to cell plate growth involves kinesin dependent phragmoplast assembly followed by cycles of phragmoplast microtubule destabilisation controlled by the conserved NACK-PQR MAP Kinase signalling cascade.

In common with NACK-PQR pathway mutants, *tio (two-in-one)* mutants initiate cell plate growth but fail to complete cytokinesis in both sporophytic and gametophytic tissues.[1] *TIO* encodes the plant orthologue of the Fused-kinase and is localised to the phragmoplast midline during cell plate expansion. *TIO* thus provides an exciting opportunity to further understand cell signalling during cell plate expansion. While Fused is widely conserved among eukaryotes from Drosophila (dFu) to Dictyostelium (TsuA), its cellular functions have evolved separately. dFu forms part of the Hedgehog signalling pathway in segment polarity and wing patterning,[2] while TsuA controls cell polarity and response to chemoattractants.[3] A common feature of Fused kinase proteins is their direct or indirect interaction with microtubules. Arabidopsis TIO does not appear to bind to Mts directly, but is associated with the phragmoplast midline and expanding phragmoplast ring during cell plate expansion.

Here we present data for the functional analysis of TIO protein domains using male gametogenesis as a single cell model to study cytokinesis *in planta*. We provide evidence that TIO-kinase activity and the regulation of TIO-kinase activity through in the C-terminal domain, are both required for completion of cell plate expansion. We will discuss the potential role of TIO in the NACK-PQR pathway, emerging evidence for specific TIO protein interactions and role of TIO in phragmoplast dynamics.

1 Oh *et al* (2005) CurrBiol 15, 2107–2111
2 Therond *et al* (1996) Genetics 142,1181-1198
3 Tang *et al* (2008) GenesDev 22,2278-2290

P376 *AtPP1* encodes a peptidyl-prolyl *cis/trans* isomerase that regulates flowering time in Arabidopsis

Yu Wang, Daiwen Yang, Hao Yu, Yih-Cherng Liou

National University of Singapore and Temasek Life Sciences Laboratory, Singapore

Floral transition in plants is regulated by an integrated network of flowering genetic pathways. We show that an Arabidopsis PIN1-type parvulin 1 (AtPP1) controls floral transition by accelerating cis/trans isomerization of the phosphorylated Ser/Thr-Pro motifs in two MADS-domain transcription factors, SUPPRESSOR OF OVEREXPRESSION OF CO 1 (SOC1) and AGAMOUS-LIKE 24 (AGL24). AtPP1 regulates flowering, which is genetically mediated by *AGL24* and *SOC1*. AtPP1 interacts with the phosphorylated AGL24 and SOC1 *in vitro* and with AGL24 and SOC1 *in vivo*, and accelerates the cis/trans conformational change *in vitro* of phosphorylated Ser/Thr-Pro motifs of AGL24 and SOC1. We further demonstrate that these Ser/Thr-Pro motifs are important for AtPP1 function in promoting flowering through AGL24 and SOC1. Thus, we propose phosphorylation-dependent prolyl cis/trans isomerization of key transcription factors as a novel flowering regulatory mechanism.

P377 Molecular chaperones and their receptors in posttranslational protein targeting

Verena Kriechbaumer, C Ottilie Freiin Loeffelholz von Colberg, Rowena Shaw, Anne-Marie Harrison, Ben M Abell

Sheffield Hallam University, Sheffield, UK

Cellular organisation depends on accurate and efficient protein targeting. Protein localisation typically exploits mechanisms based on the recognition of a signal sequence by a cytosolic targeting factor, followed by docking of the complex to a cognate receptor at the organellar outer membrane. Such a mechanism is well established for cotranslational targeting to the ER membrane, yet most targeting pathways begin after precursor synthesis i.e. posttranslational, and are initially dependent on the binding of generic cytosolic molecular chaperones such as Hsc70 and Hsp90. An initial function of molecular chaperones in protein targeting is likely to be prevention of aggregation or misfolding of the precursor. However, the recent characterisation of chaperone receptors, including TOM70 and mtOM64 at the mitochondrial membrane and TOC64 at the chloroplast membrane, suggests a more complex function for molecular chaperones. We are testing the hypothesis that the combination of chaperones in the cytosol and chaperone receptors at organellar membranes are a conserved means of contributing specificity to posttranslational targeting.

Our model system for these studies of posttranslational targeting uses tail-anchored proteins, a class of protein that is anchored to the cytosolic face of membranes by a C-terminal transmembrane span. We have performed database searches to identify tail-anchored proteins in plants and tested their localisation by microscopy and cell-free targeting assays. These proteins are being used to assess the role of chaperones in posttranslational targeting to intracellular organelles.

P378 Arabidopsis LIM domain proteins

Monika Dieterle, Clément Thomas, Jessica Papuga, Stéphane Tholl, Céline Hoffmann, Flora Moreau, André Steinmetz

CRP-Sante, Luxembourg, Luxembourg

We recently characterized the tobacco NtWLIM1 protein as an actin binding and bundling protein *in vitro* and *in vivo*. The plant actin cytoskeleton is implicated in intracellular transport, organization of the cytoplasm, and tip growth. Whereas LIM domains are widespread in animal proteins, a genome-wide analysis revealed that they occur exclusively in two different protein families of plants. One plant-specific LIM protein family contains a single LIM domain followed by a RING-like domain and frequently several N-terminal ubiquitin interacting motifs (e.g. Arabidopsis DA1). The second family contains two LIM domains, which are separated by a spacer region (e.g. NtWLIM1). We identified six such LIM-LIM domain coding sequences in the Arabidopsis genome. Analysis of their spatial and temporal expression pattern by Northern blotting, promoter-GUS studies and analysis of the public available microarray revealed that three of them are widely expressed in different tissues (WLIMs), whereas the other three are predominantly and highly present in pollen grains (PLIMs). All six associate with filamentous structures when expressed *in planta* as GFP fusion proteins, suggesting an interaction with F-actin. In contrast a GFP-DA1 fusion protein exhibited a diffuse signal in the cytoplasm. Also all six Arabidopsis WLIM and PLIM proteins can bind to and bundle F-actin *in vitro*. In order to understand their function in plant development we employ reverse genetics approaches, which didn't bear fruit yet.

P379 The exocyst in *Arabidopsis thaliana* - characterisation of Exo84 homologues

Elizabeth Turner, Steve Baldwin, Alison Baker

University of Leeds, Leeds, UK

Vesicular traffic through the endomembrane compartments and to the plasma membrane in eukaryotic cells is mediated by tethering factors. These act to initiate contact between the vesicle and target membranes, prior to the pairing of SNAREs and subsequent membrane fusion. They are crucial both for the positioning of the vesicle on the target membrane and for the specificity of the fusion event. One of these factors is the exocyst; a multisubunit protein complex bearing similarities to the COG and GARP complexes. Originally identified in yeast, the exocyst has also been studied extensively in mammalian cells, acting when physiological or developmental events require rapid exocytosis to facilitate polarised growth. Homologues of all eight of the yeast and mammalian exocyst subunits have been identified in silico in several published plant genomes, including that of *Arabidopsis thaliana*. The Arabidopsis genome apparently encodes three isoforms of *Exo84*, a subunit that plays key roles in exocyst assembly and function in yeast and mammals, which in contrast express only a single isoform. One of the plant genes may represent a pseudogene, while the other two encode proteins that exhibit 35% identity in

amino acid sequence. To build on the knowledge already gained about the exocyst in plants, the phenotypes of T-DNA insertion mutants in the *Exo84* genes have been studied. In addition, transient expression has been utilised to investigate the cellular and physiological functions of the exocyst in Arabidopsis.

P380 Arabidopsis CBP20 targets the cap-binding complex to the nucleus and is stabilized by CBP80

Daniel Kierzkowski1, Maciej Kmieciak2, Paulina Piontek2, Anna Kapczynska1, Przemyslaw Wojtaszek1, Zofia Szwejkowska-Kulinska2, Artur Jarmolowski2

1Department of Molecular and Cellular Biology, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland, 2Department of Gene Expression, Faculty of Biology, Adam Mickiewicz University, Poznan, Poland

The cap-binding protein complex (CBC) binds to the cap of all RNA polymerase II transcripts and plays an important role in RNA metabolism. We characterized interactions, localization and nuclear-cytoplasmic transport of two subunits of the *Arabidopsis thaliana* cap-binding protein complex (AtCBC), AtCBP20 and AtCBP80. Using CFP/YFP-tagged proteins, we show that transport of AtCBC from the cytoplasm to the nucleus in the plant cell is different from that described in other eukaryotic cells. We show that the smaller subunit of the complex, AtCBP20, plays a crucial role in nuclear import of AtCBC. The C-terminal part of AtCBP20 contains two functionally independent nuclear localization signals (NLSs). At least one of these two NLSs is required for import of the CBC into the plant nucleus. The interaction between the *Arabidopsis thaliana* CBP20 and CBP80 was also analyzed in detail, using the yeast two-hybrid system, Fluorescence Resonance Energy Transfer (FRET) and Bimolecular Fluorescence Complementation (BiFC) assays. The N-terminal part of AtCBP20 is essential for interaction with AtCBP80. Furthermore, AtCBP80 is important for the protein stability of the smaller subunit of CBC. Based on these data, we propose a model for nuclear-cytoplasmic trafficking of CBC complex in plants.

P381 Integrity of the plant Golgi apparatus

Carmen Faso1, Ya-Ni Chen1, Kentaro Tamura2, Aurelia Boulaflous1, Federica Brandizzi1,3

1MSU-DOE Plant Research Laboratory, Michigan State University, East Lansing 48824 (MI), USA, 2School of Life Sciences, Oxford Brookes University, Gipsy Lane, Oxford OX3 0BP, UK, 3Department of Plant Biology, Michigan State University, East Lansing 48824 (MI), USA

Unique functional characteristics of the plant Golgi are central to a wide range of biological and biotechnological processes, such as transport of building blocks to energy rich compartments, including chloroplasts, storage vacuoles and a cellulosic cell wall.

Despite its central role in plants, most of the factors that affect Golgi integrity and functionality in plant cells are yet unknown. Therefore, in response to the need for a plant Golgi-centered investigation, we developed a confocal microscopy-based screen of EMS-treated *A. thaliana* plants, which stably express the transmembrane domain and the cytosolic tail of a widely used Golgi marker fused to GFP (ST-GFP).

Our approach has led us to the isolation of several mutants exhibiting either severe mistargeting of ST-GFP to cellular compartments such as the ER and the vacuole or a visibly altered morphology of the Golgi itself. We are currently in the process of mapping these mutations to identify the genes responsible for the aberrant phenotypes.

The screen we have developed is a potential “goldmine” for the identification and characterization of novel genes or novel alleles that affect fundamental processes in the secretory pathway such as Golgi functioning and maintenance.

P382 Mechanistic framework for polar PIN targeting

Jurgen Kleine-Vehn, Jiri Friml

VIB Department of Plant System Biology, UGent Technologypark 927, 9052 Gent, Belgium

The directional transit of the phyto-hormone auxin from cell to cell plays a decisive role in determining and redefining the polarity of plant tissues (Scarpella *et al*, 2006). Moreover, spatial and temporal auxin accumulations (auxin gradients) determine positional cues for the presumptive sites of primordial development (Benkova *et al*, 2003; Friml *et al*, 2003; Reinhardt *et al*, 2003). Hence, mechanisms that guide auxin distribution and signaling represent a key to understand plant growth. The directional distribution of the phytohormone auxin depends largely on the PIN-FORMED (PIN) auxin efflux carriers that catalyze auxin transport from cell-to-cell. The coordinated polar localisation of PIN proteins to different sides of the cell determines the direction and rate of auxin flux (Wisniewska *et al*, 2006; Petrasek *et al*, 2006). Thus the dynamic and flexible nature of the polar PIN localisation regulates plant development by redefining the directional output of the auxin flux (Benkova *et al*, 2003; Kleine-Vehn *et al*, 2008). Here we present novel insights into polar PIN targeting. We used high end microscopy to address fundamental mechanisms of polar exo-, endocytosis, and reduced lateral PIN diffusion within the plasma membrane. Our data indicates that these processes jointly work to establish and maintain the robust, polar PIN deposition.

P383 Genomic and non genomic effects of auxin on PIN auxin efflux carrier trafficking

Stephanie Robert, Jurgen Kleine-Vehn, Thomek Paciorek, Jiri Friml

Department of Plant System, VIB Department of Plant System Biology, UGent Technologypark 927, 9052 Gent, Belgium

The plant signaling molecule auxin influences a remarkable variety of plant developmental processes. The current model proposes that plant cells integrate internal and external signals at the level of the polarity of auxin transport components (PIN proteins) and via the redirection of auxin fluxes which translate them into adaptive

developmental changes. It has been shown that PIN proteins are not statically localized at their polar plasma membrane domains but show constitutive recycling between the plasma membrane and endosomes (Geldner *et al*, 2001). This cycling may enable rapid changes in subcellular PIN polarity (Benková *et al*, 2003; Friml *et al*, 2002; Reinhardt *et al*, 2003; Scarpella *et al*, 2006). Recent data showed that PIN2 degradation is also a key component of the regulation of development (Kleine-Vehn *et al*, 2008, Laxmi *et al*, 2008). Interestingly, auxin is able to quickly repress the endocytosis of PIN proteins and also influences PIN protein stability. This provides a means for PIN accumulation at the plasma membrane and, thus, an essential feed-back regulation of auxin transport by auxin itself (Paciorek *et al*, 2005). The regulatory mechanisms of auxin itself on auxin transport components localization and stability will be discussed.

P384 Synthesis and trafficking of the tonoplast potassium channel AtTPK1

Marie Maitrejean1, Michael M Wudick2, Camilla Volker2, Katrin Czempinski2, Emanuela Pedrazzini1, Alessandro Vitale1

1Istituto di Biologia e Biotecnologia Agraria, Consiglio Nazionale delle Ricerche, Milan, Italy, 2Universitat Potsdam, Institut fur Biochemie und Biologie, Potsdam-Golm, Germany

Sorting signals of tonoplast proteins and the pathway they follow through the endomembrane system are still poorly characterized. To date, most studies have focused on tonoplast intrinsic proteins (TIPs) and indicated that these proteins are delivered from the endoplasmic reticulum to their destination by a Golgi-independent pathway. Is this a general pathway for tonoplast delivery? To address this question, we are studying the tandem-pore potassium channel AtTPK1. This channel has been shown to be located at the tonoplast in transient heterologous expression systems. We confirmed this localisation *in planta*, by over-expressing a TPK1-GFP fusion in transgenic Arabidopsis. Treatment with Brefeldin A, an inhibitor of Golgi-mediated secretory protein traffic, leads to mislocalization of TPK1-GFP to the endoplasmic reticulum. This indicates that the Golgi-independent pathway is not the only route for tonoplast delivery. Pulse chase analysis shows that TPK1-GFP is quite stable, with a half life of at least 24 h, and undergoes slow removal of the C-terminal, cytosolic GFP. This processing is not affected by brefeldin A, indicating that mislocalisation does not alter the stability of the fusion protein. To further investigate the role of the Golgi apparatus, we wanted to take advantage of its glycan modification properties. However, treatment with the N-glycosylation inhibitor tunicamycin indicates that the potential TPK1 N-glycosylation site at Asn131 is not used *in vivo*. We finally showed that TPK1-GFP forms homodimers. We are now investigating the relationship between TPK1 dimerisation and its sorting to the tonoplast. We generated several chimeras between TPK1 and TPK4, the homologous channels located at the tonoplast and the plasma membrane respectively. Study supported by the EU Marie Curie Research Training Network 'Vacuolar Transport Equipment for Growth Regulation in Plants' (MRTN-CT-2006–035833).

P385 Analysis of R-SNAREs mediating endosomal/vacuolar membrane fusion in *Arabidopsis thaliana*

Kazuo Ebine1, Masaru Fujimoto1, Tatsuaki Goh2, Jun Ito3, Tomohiro Uemura1, Akihiko Nakano1,4, Takashi Ueda1

1Department of Biological Sciences, Graduate School of Science, The University of Tokyo, 2Graduate School of Science, Kobe University, 3Graduate School of Biological Sciences, Nara Institute of Science and Technology, 4RIKEN, Advanced Science Institute

Post-Golgi organelles play fundamental roles in various plant functions of higher order, but our knowledge on molecular mechanisms of their biogenesis and inter-organelle traffic is still limited. We are studying the molecular mechanism of endocytosis and vacuolar transport, and how these trafficking pathways participate in plant morphogenesis with a special focus on SNARE and Rab proteins. We previously screened all SNARE proteins for endosomal SNAREs, and found that only one R-SNARE protein, VAMP727, localized almost exclusively on the RAB5/RABF-positive endosomes (Ueda *et al*, 2004; Uemura *et al*, 2004). The subcellular localization suggested that VAMP727 is involved in membrane fusion at the endosomes.

AtVAM3/SYP22 is a Qa-SNARE localizing on the vacuolar membrane and the pre-vacuolar compartment (PVC), and is reported to form a stable complex with VTI11 and SYP5. However, the R-SNARE in this SNARE complex has remained undiscovered. Through genetic, live imaging, and biochemical approaches, we have revealed that VAMP727 is the R-SANRE comprising the SNARE complex with VAM3, VTI11, and SYP51 on the PVC/endosome closely associated with the vacuolar membrane (Ebine *et al*, 2008).

We have also shown that another R-SNARE, VAMP711-713 is localized to the vacuolar membrane (Uemura *et al*, 2004). We recently found that VAMP713 also forms a complex with VAM3, VTI11 and SYP5. In this meeting, we will report our recent progress on the regulatory mechanism of these two VAM3 complexes formation.

P386 Influence of reduced UDP-glucose dehydrogenase activity on *Arabidopsis thaliana*: New cell wall mutants

Rebecca Reboul, Claudia Geserick, Ursula Lütz-Meindl, Raimund Tenhaken

University of Salzburg, (Salzburg), Austria

The plant cell wall has the determinant role to provide skeletal support to the plant itself.

As a complex polysaccharide structure, many aspects remain unclear: the formation of the cell wall, its 3D-model or the role of the unique elements is still not completely understood. Studying cell wall mutants contributes to better understand this fundamental structure.

Here we focus our interest on single and double *ugd* mutants, which show a knockout in either one or two isoforms of UDP-glucose dehydrogenase (UGD). This particular enzyme uses UDP-glucose to form

UDP-glucuronic acid, which has a key role in the synthesis of different nucleotide-sugars (UDP-arabinose, -apiose, -galacturonic acid, -xylose) composing 50% of the cell wall biomass.

All single mutants and almost all double mutants *(ugd1,4; ugd1,3; ugd1,2)* have a quite consistent phenotype: hypocotyls and single cells are slightly longer in comparison to the wild-type and TEM analyses of *ugd1,4* revealed thinner and stretched cell walls. Surprisingly, no difference in cell wall crude composition of adult plants is notable. On the other hand, *ugd2,3* double mutants, lacking two isoforms with high expression levels in the first stages of root formation, shows a dwarfed phenotype. Development defects in seedlings are quite pronounced, the cell wall looks swelled and is twice as thick as the WT one; sugar composition and metabolites of *ugd2,3* are disturbed. The use of monoclonal antibodies against cell wall substructures and treatments with specific cell wall digesting enzymes give some explanations to the observed phenotype. Pectins and xyloglucans are affected because of a UDP-sugar imbalance in the mutant compared to wild-type.

UGD isoforms do not have the same importance and role for the seedling. A slightly reduced activity of UGD leads to an elongation of the cell and thinning of the cell wall. A stronger reduction in UDP-glucuronic acid supply below a critical threshold causes dwarfism and strong developmental defects.

P387 A tight balance between cyclin-dependent kinases and their inhibitors controls male gametophyte development in Arabidopsis

Xin'ai Zhao1, Hirofumi Harashima1, Nico Dissmeyer1,2, Stefan Pusch2, Nicolas Baumberger1, Moritz K Nowack2, Pascal Genschik1, Arp Schnittger1,2

1Institut de Biologie Moleculaire des Plantes du CNRS IBMP-CNRS - UPR2357, 67084 STRASBOURG ,France, 2Max Planck Institute for Plant Breeding Research, 50829 Koln, Germany

Cyclin-dependent kinases are the key regulators of cell cycle control in all eukaryotes. Dominant negative kinase versions have been an important tool to address CDK function and regulation during development since they typically block cell proliferation by titrating cyclins or kinase substrates. However, here we show that a dominant negative kinase version of the Arabidopsis *cdc2/CDC28* homolog CDKA;1 can also titrate negative cell cycle regulators, i.e. CDK inhibitors of the ICKs/KRPs class, and thus, could promote cell cycle progression. Introduction of this construct into a *cdka;1* mutant background could rescue the cell cycle arrest of *cdka;1* mutants during male germ line development, i.e. promote the division of a generative cell into two sperms that is missing in *cdka;1* mutants. This indicates that the ratio between CDK inhibitors and CDKA;1 is crucial for the formation of two sperms. The unique properties of the dominant negative CDK version will be further exploited to unravel regulatory cascades during male germ line development and plant growth.

P388 Characterization of the *LEA18* gene family of *Arabidopsis thaliana*

Margarita Santiago, Maria Patricia Romero, Dayan Sanhueza, Herman Silva

Universidad Andres Bello, Santiago, Chile

To identify genes involve in the plant secretory pathway we screened for *Arabidopsis thaliana* cDNA clones that complement temperature-sensitive exocyst mutants of *Saccharomyces cerevisiae.*

AtLEA18-2 suppressed exocyst mutants growth and secretory defects. This gene shows no sequence similarity to the exocyst components, but encodes a protein of the Arabidopsis *LEA18* gene family. This is a newly described group which has three components in Arabidopsis that are similar to *Phaseolus vulgaris* PvLEA-18 gene, all of them with unknown function.

In order to study the gene function of the 3 components of the *LEA18* family we analyzed gene expression by qPCR in different plant tissues (caulinar and rossette leaves, stems, flowers, buds, roots, siliques and seeds). It was observed that *AtLEA18-2* expression is ubiquitous, however *AtLEA18-1* is expressed in green siliques and seeds and *AtLEA18-3* is expressed only in buds.

Currently we are analyzing the importance of the genes 3´region, because it is know that it has an enhancer effect in PvLEA18. We also obtained an insertional mutant line in *AtLEA18-2*. A homozygous plant for the insertion does not show transcript accumulation. On the other hand, we observe ectopic expression of its homologue *AtLEA18-1*, suggesting gene redundancy. We obtained the double mutant (*Atlea18-1/Atlea18-2*) without an apparent phenotype.

At this moment we are subjecting the mutants to different stresses to identify possible conditional phenotype in the single and double mutant. The triple mutant (*Atlea18-1/Atlea18-2/Atlea18-3*) has a 25% of non-viable pollen and produced 50% fewer seeds than a wild type plant. This could suggest an important role in gametes development.

This work is financed by Millennium Nucleus in Plant Cell Biology and Biotechnology PCB P06-065-F.

P389 The Dof family of plant transcription factors: Insights into the evolution of non-cell-autonomous function

Munawar Ahmad1, Yeonggil Rim1, Won Kyong Cho1, Hyosub Chu1, Yeonhwa Jo1, Xuping Zhao1, Che Ok Jeon2, Hye-Jin Kim1, Jong-Chan Hong1, William J Lucas3, Jae-Yean Kim1

1Division of Applied Life Science, Environmental Biotechnology National Core Research Center, Plant Molecular Biology and Biotechnology Research Center, Gyeongsang National University, Jinju 660-701, Korea., 2Chung-Ang University, Dept of Life Science, 221, HeukSeok-Dong, Dongjak-Gu, Seoul, 156-756, Korea, 3Dept of Plant Biology, College of Biological Sciences, University of California, Davis, California 95616, USA

Dof proteins are plant-specific transcription factors that are involved in a myriad of functions. The highly conserved "Dof domain" characteristic of this family performs the dual function of DNA binding and protein-protein

interaction. Members of this family appear to have played an important role in the evolution of land plants. We isolated Dof4.1as a non-cell-autonomous transcription factor (NCATF) in a genome-wide screen using the GAL4-UAS activation system in Arabidopsis. For this purpose, we used the CS9094 GAL4 enhancer trap line which drives the expression of GFPer in the cortex and endodermal layers of root tip. A Dof4.1:mCherry fusion protein was found to move out from the cortex/endodermis region of root into the adjacent cell layers. The trichome rescue-trafficking assay was employed to establish that Dof4.1 traffics selectively through plasmodesmata, conferring gain-of-trafficking function to the cell-autonomous GLABROUS1 (GL1) protein. Transcriptional fusion with the GUS reporter system indicated that Dof4.1 is expressed primarily within the vasculature system and apical meristems of the seedling. Parallel molecular analyses are being performed on Dof4.1 and equivalently-sized cell-autonomous Dof proteins in order to identify the domains(s) responsible for conferring non-cell-autonomous function. These findings will enable us to further investigate the evolution and function of the Dof family of proteins as transcriptional regulators in the plant kingdom.

Supported by EB-NCRC,BK21 and WCU program grants

P390 Metabolomic analysis of Arabidopsis suspension cultured cells that overexpress a putative vacuolar membrane transporter

Koh Aoki1, Ryosuke Sasaki1,2, Miwa Ohnishi3, Aya Anegawa3, Yuko Sugiyama4, Yoko Iijima1, Nozomu Sakurai1, Mami Yamazaki5, Tetsuro Mimura3

1Kazusa DNA Research Institute, Kisarazu, Japan, 2CREST, Tokyo, Japan, 3Kobe University, Kobe, Japan, 4University of Hyogo, Himeji, Japan, 5Chiba University, Chiba, Japan

The vacuole of a plant cell is a multi-functional organelle that contributes to cell growth, storage of ions, and storage of various types of metabolites. Proteomic analysis revealed that there are more than 160 proteins on the tonoplast membrane, including 34 unknown trans-membrane proteins that putatively contribute to the transport of metabolites across the tonoplast membrane (Shimaoka *et al*, 2004). However, actual substrates of these putative transporters have not been clarified. To identify substrates of the unknown vacuolar transporters, we are producing a series of transgenic lines each of which overexpresses a gene encoding one of the transporters, and analyzing metabolite profiles of the cell and isolated vacuoles. In this study, we report metabolic profiling of a transgenic line of Arabidopsis suspension cultured cells that overexpresses a gene encoding a MATE efflux family protein, *At3g21690*, and a comparative analysis with *wild type (wt)* cells. Analysis of whole-cell extract demonstrated that the accumulation levels of amino acids and flavonols increased, and that the accumulation levels of glutathione decreased. Additionally, significant changes were observed in accumulation levels of several unannotated metabolites. On the other hand, overexpression of *At3g21690* did not alter the levels of nucleotides. Comparison with the metabolite annotations of the vacuole isolated from *wt* Arabidopsis cultured cell implies that flavonols and amino acids are candidates of substrate of this MATE efflux family protein. The results will be discussed in conjunction with the transcript analysis of the transgenic cells. This work was partly supported by CREST, JST.

P391 Isolation and identification of ubiquitin-related proteins from Arabidopsis seedlings

Tomoko Igawa1, Masayuki Fujiwara1, Hirotaka Takahashi2, Tatsuya Sawasaki2, Yaeta Endo2, Motoaki Seki3, Kazuo Shinozaki3, Yoichiro Fukao1, Yuki Yanagawa1

1The Plant Science Education Unit, The Graduate School of Biological Sciences, Nara Institute of Science and Technology, Nara, Japan, 2Cell-Free Science and Technology Research Center, Ehime University, Matsuyama, Japan, 3RIKEN Bioresource Center, Tsukuba, Japan

The majority of proteins in eukaryotic cells are modified according to highly regulated mechanisms to fulfill specific functions and to achieve localization, stability, and transport. Protein ubiquitination is one of the major post-translational modifications occurring in eukaryotic cells. To obtain the proteomic dataset related to the ubiquitin (Ub)-dependent regulatory system in Arabidopsis, affinity purification with an anti-Ub antibody under native condition was performed. Using MS/MS analysis, 196 distinct proteins represented by 251 distinct genes were identified. The identified proteins were involved in metabolism (23.0%), stress response (21.4%), translation (16.8%), transport (6.7%), cell morphology (3.6%), and signal transduction (1.5%), in addition to proteolysis (16.8%) to which proteasome subunits (14.3%) is included. On the basis of potential ubiquitination-targeting signal motifs, in-gel mobilities, and previous reports, 78.0% of the identified proteins were classified as ubiquitinated proteins and the rest were speculated to be associated proteins of ubiquitinated proteins. The degradation of three proteins predicted to be ubiquitinated proteins was inhibited by a proteasome inhibitor, suggesting that the proteins were regulated by Ub/proteasome-dependent proteolysis.

P392 Analysis of interactions between Arabidopsis CAX1 and CAX3 Ca^{2+} transporters

James Connorton1, Jian Zhao2, Ying-qing Guo2, Toshiro Shigaki2, Kendal Hirschi2, Jon Pittman1

1University of Manchester, Manchester, UK, 2Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX, USA

The Arabidopsis vacuolar H+/Ca^{2+} exchangers CAX1 and CAX3 efflux Ca2+ from the cytosol to provide Ca^{2+} tolerance and reset cytosolic Ca^{2+} levels after a signalling event. Efficient regulation of their activity is crucial as deregulation of these transporters has deleterious consequences for the plant. The expression profiles of *CAX1* and *CAX3* are distinct in mature plants with high expression in shoot and root tissue respectively. However under defined conditions and in the young seedling *CAX1* and *CAX3* expression overlaps. Previous studies have

provided evidence for functional association of CAX1 and CAX3, however, little is currently known about the nature of these putative protein-protein interactions. We have analysed the interactions between full-length CAX1 and CAX3 proteins and between CAX protein domains. *In vivo* bimolecular fluorescence complementation was used to confirm interaction and co-localisation of CAX1 and CAX3. Many transporter proteins including CAX transporters are made up of two or more homologous domains, possibly as a result of ancient duplication events. Analysis of the interactions of separately expressed halves of CAX1 and CAX3 showed that the C-terminal halves of both proteins could interact with the N-terminal half of CAX1 to form a functional reconstituted Ca2+ transporter. The CAX1-CAX3 interaction may be an important mechanism by which Arabidopsis controls cytosolic Ca^{2+} levels in response to certain stimuli. Furthermore, these studies will provide insight into the structure of oligomeric CAX complexes.

P393 Analysis of the protein association of the ribosomal lateral stalk by immunoprecipitation of epitope tagged 12-kDa P-proteins

Luz Maria Borreli, Kathleen Szick-Miranda
California State University, Bakersfield, USA

The synthesis of proteins is an absolute requirement for growth and development in biological organisms, yet our understanding of the ribosome and individual ribosomal proteins (r-proteins) in plants remains limited. Specifically, we have focused on the 12-kDa acidic ribosomal phosphoproteins (P-proteins) which form a universally conserved lateral stalk in the active site of the large ribosomal subunit and are thought to assist in the late initiation and elongation phases of translation via interactions with tRNA, mRNA and translation factors. Higher plant ribosomes possess evolutionarily conserved (P1, P2a and P2b) and evolutionarily distinct (P3) forms of 12-kDa P-proteins. Additionally, the presence, abundance and phosphorylation of these proteins in maize ribosomes varies during development. We hypothesize that the heterogeneity observed with these proteins plays a role in the regulation of gene expression via selective mRNA translation. Our goals are to determine the organization of acidic ribosomal P-protein stalk and to elucidate the functional significance of ribosome heterogeneity with respect to the acidic ribosomal P-proteins in Arabidopsis. We plan to examine the specific composition of the stalk by immunoprecipitation of individual epitope tagged P-proteins and mass spectrophotometric (MALDI-TOF) analysis of co-precipitated proteins. Lastly, protein synthesis of mRNAs extracted from immunoprecipitated ribosomes of known stalk composition will be examined to determine if cytosolic ribosomes with varying P-protein stalk composition, translate a different set of mRNAs. Our analyses will add significantly to the understanding of the protein constitution of plant ribosomes and the functional significance of ribosome heterogeneity. Furthermore, P-protein genes (including the plant specific P3) are also maintained in important crop species indicating that Arabidopsis is an excellent model system to study genes that may be of agronomic importance. Together, these data will provide a landmark for the analysis of higher plant ribosomes and has the potential to widely impact agriculture through the prospect of higher crop yield.

P394 Synthesis of very long chain fatty acids in the epidermis controls cell division in Arabidopsis

Takashi Nobusawa, Yoko Okushima, Masaaki Umeda
Graduate School of Biological Sciences, Nara Institute of Science and Technology

The epidermis is an essential interface against outer circumstances, especially cuticle, which covers the aerial part of vascular plants and some bryophytes, functions as a hydrophobic barrier under various environmental conditions. Plant cuticle consists of cutin matrix and epicuticular wax that are synthesized from very long chain fatty acids (VLCFAs) and their derivatives (i.e. VLCFA esters and ketones). VLCFAs are also used to synthesize seed triacylglycerols, sphingolipids and phospholipids. However it is still unknown whether they have a regulatory function in the continuous development of plants.

PASTICCINO2 (PAS2) was identified from an Arabidopsis mutant that showed hypersensitivity to cytokinin. *pas2* mutants exhibit pleiotropic phenotypes in aerial tissues, such as organ fusion and curled leaves as well as severe growth defects. Recently Bach *et al* (2008) reported that *PAS2* encodes 3-hydroxy-acyl-CoA dehydratase that is an essential and limiting enzyme in VLCFA synthesis. We found that Arabidopsis seedlings treated with a VLCFA synthesis inhibitor displayed ectopic cell division in the shoot apical meristem, young leaves and hypocotyls, suggesting that inhibition of VLCFA synthesis disorders cell proliferation during organ formation. When *PAS2* was specifically expressed in the epidermis, it fully rescued developmental defects observed in *pas2* mutants. In contrast, down-regulation of *PAS2* in the epidermis of wild-type seedlings caused *pas2*-like phenotypes. These results indicate that VLCFA synthesis in the epidermis controls aerial organ development via a non-cell autonomous pathway.

Bach *et al* (2008) The very-long-chain hydroxy fatty acyl-CoA dehydratase PASTICCINO2 is essential and limiting for plant development. Proc. Natl. Acad. Sci. USA, 105, 14727-14731.

P395 Cohesin gene defects impair sister chromatid alignment and genome stability in *Arabidopsis thaliana*

Veit Schubert, Andrea Weissleder, Hoda Ali, Inna Lermontova, Joerg Fuchs, Ingo Schubert
Leibniz Institute of Plant Genetics and Crop Plant Research, D-06466 Gatersleben, Germany

In contrast to yeast, sister chromatids are often not completely aligned along chromosome arms in interphase nuclei of higher plants, whereas sister centromeres stay mostly aligned until an endopolyploidy level of 16C (Schubert *et al* 2006, Genetics 172, 467-475; Schubert *et al* 2007, Mol. Genet. Genomics 278, 167-176). Sister

chromatid cohesion (alignment) mediated by the multi-subunit complex cohesin is essential for correct chromosome segregation during mitosis and meiosis and also for DNA recombination repair. The cohesin complex consists of the conserved proteins SMC1, SMC3, SCC3 and an α-kleisin subunit. *Arabidopsis thaliana* has four α-kleisin genes named *Syn1, Syn2, Syn3* and *Syn4*.

To analyse the consequences of "knocking out" separately all potential Arabidopsis cohesin genes on interphase sister chromatid alignment, differentiated 4C nuclei of T-DNA insertion mutants were evaluated after fluorescence *in situ* hybridization (FISH) with ~100 kb probes from chromosomal mid-arm positions and with the centromeric 180 bp-repeat.

Viable homozygous mutants could be selected only for *Syn1, Syn2,* and *Syn4*. Thus, these α-kleisin genes can partially compensate each other. For *Syn3* and for the single-copy genes *Smc1, Smc3* and *Scc3* only heterozygous mutants showing >50% of wild-type mRNA expression level were obtained. Thus, these genes are essential for plant viability. Compared to wild-type nuclei, FISH revealed a significantly decreased sister chromatid alignment at ~100 kb mid-arm segments in homozygous *syn1* and *syn4* mutants and even in the heterozygous *smc1*, *smc3, scc3* and *syn3* mutants. Furthermore, "knocking out" *Syn1* and *Syn4* impairs sister centromere cohesion. A high frequency of anaphase bridges in *syn2, syn3* and *swi1* mutants indicates decreased genome stability in these lines.

P396 Function of NimA-related protein kinases in Arabidopsis

Hiroyasu Motose1, Takahiro Hamada2, Takehide Kato2, Takashi Hashimoto2, Yuichiro Watanabe3, Tatsuya Sakai4, Taku Takahashi1

1Division of Bioscience, Graduate School of Natural Science and Technology, Okayama University, Okayama, Japan, 2Graduate School of Biological Science, Nara Institute of Science and Technology, 3Graduate School of Arts & Sciences, The University of Tokyo, 4Plant Science Centre, RIKEN

NimA-related kinases (NEKs) are highly conserved in all eukaryotes and extend into multi-gene family in animals and plants. Previous studies in fungi and animals indicated that NEKs regulate mitotic entry and progression, centrosomal separation, spindle formation, and cilia function, while functions of higher plant NEKs remain to be elucidated. Our recent work revealed that Arabidopsis NEK6 modulates the directional cell growth and morphogenesis interacting with microtubles and armadillo-repeat kinesins (ARKs) (Sakai *et al* Plant J. 53, 157-171, Motose *et al* Plant J. 54, 829-844). In this report, we describe functional analysis of NEK family in Arabidopsis with special emphasis on NEK6. NEK6 was highly accumulated in meristematic cells and expanding cells. NEK6 associates with the cytokinetic phragmoplasts in dividing cells and cortical microtubules in expanding cells. NEK6 concentrates in dot-like structures exhibiting dynamic movement along with the microtubules. NEK6 phosphorylates microtubule-related proteins and modulates directionality of cortical microtubule array. NEK6 co-localizes with other members of NEK family and regulates directional cell growth and morphogenesis.

P397 Functional analyses of cell wall genes involved in physical properties of supportive tissue in the Arabidopsis stem

Ryusuke Yokoyama, Kaori Satz, Kento Koizumi, Miki Nogami, Kazuhiko Nishitani

1Department of Developmental Biology and Neurosciences, Graduate School of Life Sciences, Tohoku University, Sendai, 980-8578 Japan

In *Arabidopsis thaliana*, rapid elongation of the inflorescence stem occurs during reproductive growth. The inflorescence stem functions not only to support the elevation of flowers and fruits, but also to hold its own weight. The supportive tissue of the inflorescence stem consists of several distinct types of cells with specialized cell wall structures. The specific structural features of individual cell walls confer mechanical strength to the inflorescence stem. Recently, significant progress has been made in identifying the cell wall genes responsible for the mechanical strength of plant tissues. However, our present knowledge is still insufficient to account for the molecular processes by which this complex system operates. To acquire further information about the cell walls responsible for mechanical strength, we have focused our research effort on a set of genes that are specifically implicated in cell wall formation in the supportive tissue of the inflorescence stem. Results from two microarray screenings identified several key candidate cell wall genes responsible for the mechanical strength of the supportive tissue. We examined expression profiles of promoter::GUS fusion constructs for each of the genes. The results indicate that most of the genes were expressed in xylem and/or interfascicular region where secondary wall thickening occurred, whereas some genes have a distinct cell-type-specific expression profiles in base of the stem. Expression of the glycine-rich protein (*GRP*) gene was restricted to xylem parenchyma cells, and one of the pectin methylestrase (*PME*) genes was expressed mainly in the cortex. To identify the role of the individual gene, we also examined phenotypes of mutants in which each of the genes was disrupted. The loss-of-function mutation of the *PME* gene displayed several morphological phenotypes in the inflorescence stem. Based on these findings, we discuss the roles of the cell wall genes involved in physical properties of the inflorescence stem.

P398 Determining primary and specific effects on the nuclear transcriptome in response to inhibition of plastid translation in Arabidopsis

Katharine Howell, Ralph Bock

Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany

Retrograde regulation refers to the phenomenon whereby signals emanating from the plastids or the mitochondria, reflecting their functional and developmental state, exert a regulatory effect on nuclear genes, often encoding organellar-targeted proteins. While this process is crucial to plant growth and survival, the exact molecular mechanisms underlying this form of regulation are not well understood. With respect to plastid-to-nucleus retrograde signalling, it

has been proposed that this form of communication involves: plastid gene expression, redox signals, the tetrapyrrole pathway and/or reactive oxygen species. The research presented focuses on the signals generated by plastid translation using a controlled pharmacological approach in Arabidopsis. While previous studies have sought to define the nuclear genes targeted by these pathways a clear picture has not yet emerged given that former experiments often involve long treatments, introducing secondary effects, and use of inhibitors, which often have non-specific effects. Thus, dissecting out the target genes directly responsive to the retrograde signal is difficult. To address these issues we are comparing wild-type Arabidopsis with a spectinomycin resistant line so non-specific effects of the plastid translation inhibitor, spectinomycin, can be taken into account. Furthermore, changes in the transcriptome due to secondary effects are reduced by sampling at early timepoints after inhibition. Using this system, transcriptomic analysis has been performed using microarrays and qRT-PCR and has identified putative regulatory components of the chloroplast gene expression retrograde signal. Those discovered provide direction for future studies with the ultimate long-term aim to identify all the components involved in this elusive signalling pathway.

P399 StSEBF-homologs in *Arabidopsis thaliana*

Janina Fuss, Kirsten Krause

Department of Biology, University of Tromsø, NO-9037 Tromsø, Norway

The SEBF-protein found in potato has been shown to be involved in defence reactions by binding to the ERE (Elicitor Response Element) of the *PR-10a* gene (Boyle and Brisson, 2001). SEBF in potato is a mediator of the assembly of several transcriptional regulators in the nucleus to repress the expression of *PR-10a* (Gonzalez-Lamothe *et al*, 2008). In Arabidopsis, homologues of SEBF have been shown to be localized to the chloroplast (Ohta *et al*, 1995). Another study describes the same protein without targeting signal as a nucleolin like (and therefore nucleus-localised) protein (Didier and Klee, 1992) Furthermore, they bind poly-U stretches of RNA. Their localisation in chloroplasts and nucleus and their DNA-/RNA-binding abilities make them very interesting candidates for retrograde signalling as a reaction on wounding between chloroplast and the nucleus to adjust gene expression in those two of three DNA-containing compartments. We have selected 4 members from the SEBF-like protein family from Arabidopsis and have performed analysis of their intracellular localisation. In addition, knock-out mutants have been analysed for their phenotype.

Boyle B, Brisson N (2001) Repression of the defense gene PR-10a by the single-stranded DNA binding protein SEBF. Plant Cell 13: 2525-2537

Didier DK, Klee HJ (1992) Identification of an Arabidopsis DNA-binding protein with homology to nucleolin. Plant Mol Biol 18: 977-979

Gonzalez-Lamothe R, Boyle P, Dulude A, Roy V, Lezin-Doumbou C, Kaur GS, Bouarab K, Despres C, Brisson N (2008) The transcriptional activator Pti4 is required for the recruitment of a repressosome nucleated by repressor SEBF at the potato *PR-10a* gene. Plant Cell 20: 3136-3147

Ohta M, Sugita M, Sugiura M (1995) Three types of nuclear genes encoding chloroplast RNA-binding proteins (cp29, cp31 and cp33) are present in *Arabidopsis thaliana*: presence of cp31 in chloroplasts and its homologue in nuclei/cytoplasms. Plant Mol Biol 27: 529-539

P400 Drought stress-induced Rma1H1, a RING membrane-anchor E3 ubiquitin ligase homolog, regulates aquaporin levels via ubiquitination in transgenic Arabidopsis plants

Hyun Kyung Lee1, Seok Keun Cho2, Ora Son2, Zhengyi Xu1, Inhwan Hwang1, Woo Taek Kim2

1POSTECH, Pohang, Korea, 2Yonsei University, Seoul, Korea

Ubiquitination is involved in a variety of biological processes, but the exact role of ubiquitination in abiotic responses is not clearly understood in higher plants. Here, we investigated Rma1H1, a hot pepper (Capsicum annuum) homolog of a human RING membrane-anchor 1 E3 ubiquitin (Ub) ligase. Bacterially expressed Rma1H1 displayed E3 Ub ligase activity *in vitro*. *Rma1H1* was rapidly induced by various abiotic stresses, including dehydration, and its overexpression in transgenic *Arabidopsis thaliana* plants conferred strongly enhanced tolerance to drought stress. Colocalization experiments with marker proteins revealed that Rma1H1 resides in the endoplasmic reticulum (ER) membrane. Overexpression of *Rma1H1* in Arabidopsis inhibited trafficking of an aquaporin isoform PIP2;1 from the ER to the plasma membrane and reduced PIP2;1 levels in protoplasts and transgenic plants. This Rma1H1-induced reduction of PIP2;1 was inhibited by MG132, an inhibitor of the 26S proteasome. Furthermore, Rma1H1 interacted with PIP2;1 *in vitro* and ubiquitinated it *in vivo*. Similar to Rma1H1, Rma1, an Arabidopsis homolog of Rma1H1, localized to the ER, and its overexpression reduced the PIP2;1 protein level and inhibited trafficking of PIP2;1 from the ER to the plasma membrane in protoplasts. In addition, reduced expression of Rma homologs resulted in the increased level of PIP2;1 in protoplasts.

We propose that Rma1H1 and Rma1 play a critical role in the downregulation of plasma membrane aquaporin levels by inhibiting aquaporin trafficking to the plasma membrane and subsequent proteasomal degradation as a response to dehydration in transgenic Arabidopsis plants.

P401 AtBG2, a ß-glucosidase homolog localized in the vacuole increases cellular ABA via hydrolysis of ABA-GE

Zhengyi Xu

Postech, Department of Life Science, Nam-Gu Pohang, Korea

Abscisic acid (ABA) is a phytohormone critical for various cellular responses including germination, growth and

adaptation to the enviromental changes. Recently, generation of ABA through hydrolysis of ABA-GE by AtBG1 contributes to the increase of cellular ABA levels. Here we report isolation of AtBG2, a new vacuolar b-glucosidase homolog that affects expression of an osmotic stress responsive gene. The expression of AtBG2 was high in specific cell types including veins of leaf and cotyledon, hydathodes and tricombs of leaf, vascular bundles of hypocotyl and roots, and induced by dehydration stress. Immunopurified AtBG2:HA hydrolyzes ABA-GE to ABA *in vitro*. AtBG2 existed in the vacuole as a high molecular weight complex. Dehydration stress causes its accumulation to high levels by inhibiting proteolysis. Loss-of-function mutants, *atbg2*, displayed defects in NaCl and dehydration stress responses.

In contrast, *AtBG2* overexpression causes enhanced resistance to dehydration stress and increases ABA contents in seeds.

Based on these observations, we propose that AtBG2 localized to the vacuole plays a critical role in increasing cellular ABA levels upon dehydration.

P402 AtENT1, an adaptor for clathrin-mediated endocytosis, may play a role in the endocytic pathway during plant cytokinesis

Kyungyoung Song, Hyangju Kang, Eunhye Kwon, Inhwan Hwang

POSTECH, pohang, South Korea

Plant somatic cytokinesis starts in the center of a dividing cell with the *de novo* formation of a disk-shaped membrane compartment, the cell plate then grows outward and fuses with the parental plasma membrane. This process requires two components, a specific cytoskeletal array for vesicle delivery and vesicle fusion machinery. Although the vesicle trafficking from the TGN to the cell plate is known as the default pathway of newly synthesized components to maturing cell wall and membrane, the function of the endocytic pathway is pooly understood during cytokinesis.

Here, we found that the putative endocytic adaptor (AtENT1) is localized not only the plasma membrane in non-dividing cells but also the cell plate in dividing cells. AtENT1 has an AP180 N-terminal homolog (ANTH) domain that is known to interact with PI(4,5)P2 enriched in the plasma membrane. The ANTH domain of AtENT1 also interact with PI(4,5)P2. This indicates that AtENT1 may be bind to the plasma membrane by binding to PI(4,5)P2. Protein interaction experiments showed that AtENT1 interacts with clathrin and α-adaptin of AP-2.

Furthermore, AtENT1:sGFP in transgenic plants localizes to the cell plate during cytokinesis and *AtENT1* RNAi transgenic plants displayed the growth defect. Based on these results, we propose that AtENT1 plays an important role in endocytosis through interactions with α-adaptin, clathrin, and PtdIns(4,5)P2 during cytokinesis.

P403 Characterization of DAD1-like acylhydrolase related to seed viability in Arabidopsis

Eun Yu Kim, Young Sam Seo, Soo-Jin Kim, Woo Taek Kim

Yonsei University, Seoul, Korea

Lipid-derived molecules produced by acylhydrolases play important roles in the regulation of diverse cellular functions in plant signaling. In Arabidopsis, DAD1-like acylhydrolase family consists of 12 genes, which possesses lipase 3 domain (Pfam accession no. PF01764). In this study, we investigated biochemical and cellular functions of DAD1-like acylhydrolase encoded by At1g30370. To examine whether this protein contains acylhydrolase activity, At1g30370 protein lacking the N-terminal 114 amino acid residues, including a putative transit peptide, was expressed in *E. coli* as a fusion protein with maltose-binding protein. *In vitro* biochemical assay revealed that At1g30370 can catalyze the hydrolysis of 4-nitrophenyl butyrate, an artificial substrate for acylhydrolase, suggesting that At1g30370 is an acylhydrolase. The optimal conditions for At1g30370 activity were estimated to be pH 7.0~8.0 with Triton X-100. The fluorescent microscope analysis revealed that At1g30370 protein targets to mitochondria *in vivo* as predicted by TargetP program. Gene expression study performed by real-time PCR showed that At1g30370 gene was highly expressed in seedling. The phenotype analysis of At1g30370 - overexpressing transgenic plants and knockout mutant revealed that At1g30370 overexpressors were germinated earlier than wild type plants at normal condition and more tolerant to aging treatment. On the other hand, the knockout mutants of At1g30370 showed lower germination level and were susceptible to accelerated aging. These results suggest that the At1g30370 has an acylhydrolase activity and plays an important role in seed viability and longevity in Arabidopsis.

P404 Tail-anchored proteins in plants

Emanuela Pedrazzini

Istituto di Biologia e Biotecnologia Agraria, CNR, Milano, Italy

Tail-anchored (TA) proteins are a class of polypeptides integrated into the membrane by a C-terminally located hydrophobic sequence. TA proteins lack an N-terminal signal peptide and reach their destination within the cell by post-translational mechanisms. They are present in each of the three domains of life, performing a variety of essential functions on the cytosolic face of cellular membranes and, in several cases, determining organelle identity. A number of TA proteins insert directly into the lipid bilayer without the help of molecular machineries, suggesting that they may be ancestral proteins able to recruit lipids, contributing to the formation of intracellular compartments during cell evolution. Here, a bioinformatic approach was used to produce a catalogue of putative TA proteins encoded by the *Arabidopsis thaliana* genome, and intracellular localization was predicted based on features of well characterized TA proteins. A recent strategy aimed at improving the accumulation of recombinant proteins expressed in transgenic plants is also described. Work supported by the projects EU FP6 ‘Pharma-Planta’ [LSH-2002-1.2.5-2] and MIUR PRIN 20073YHRLE.

P405 AtKCO3 potassium channel and tonoplast biogenesis

Giuseppe Grassi1, Alexandra Grippa1, Alessandro Vitale1, Katrin Czempinski2, Emanuela Pedrazzini1

1Istituto di Biologia e Biotecnologia Agraria CNR, Milano, Italy, 2University of Potsdam, Golm, Germany

Plant cell homeostasis is maintained by the activity of channels and transporters. These proteins must be specifically targeted, sorted and retained at appropriate membrane domains to control the vectorial transport of fluid, solutes, and electrolytes. The extent of permanence at the site of action could also be regulated, through interactions with the cytoskeleton or other associated proteins. Therefore, targeting signals as well as signals that control turnover could coexist on the polypeptide. We are studying AtKCO3 potassium channel as a model to identify targeting and turnover signals and possible interactors. AtKCO3 is a single pore channel with two transmembrane domains and the N- and C-terminal regions exposed in the cytosol. A 14-3-3 binding region and two EF-hands are predicted at the N- and C-terminal domains, respectively. An AtKCO3::GFP fusion was previously found to be located at the tonoplast by transient expression. By subcellular fractionation, we confirmed the tonoplast localization of wild type AtKCO3 and the AtKCO3::GFP fusion in Arabidopsis transgenic plants. We also determined that AtKCO3::GFP forms dimers, and not tetramers as predicted. This indicates that the C-terminal GFP fusion could interfere with the channel biogenesis. We identified a putative PDZ-binding motif of class 1 (-X-S/T-X-Hydrophobic) at the C-terminus of AtKCO3 (-ATSV). PDZ proteins act as adaptors that facilitate signaling or determine the localization of receptors, channels, transporters and other signalling molecules. Preliminary experiment showed that the deletion of this motif enhances the stability of AtKCO3. This suggests a role of PDZ proteins in determining the turnover and half-life of AtKCO3. Study supported by the EU Marie Curie Research Training Network 'Vacuolar Transport Equipment for Growth Regulation in Plants' (MRTN-CT-2006–035833).

P406 A constitutively active phyA mutant is deficient in nuclear transport

Anja Possart, Wiebke Nordmeier, Eberhard Schaefer, Andreas Hiltbrunner

University of Freiburg, Germany

Phytochromes (phyA-E in Arabidopsis) are red/far-red photoreceptors, which play an important role throughout the life cycle of plants. phyA is essential for perception of far-red light whereas phyB is the major red light receptor. Phytochromes localise to the cytosol in the dark but accumulate in the nucleus after light-induced conversion to the biologically active form. Although nuclear transport of the photoreceptor itself is a key step in phyA and phyB signalling the two photoreceptors use different mechanisms for translocation into the nucleus. We could show that FHY1 and FHL are essential for phyA nuclear transport and that they interact with phyA in a light-regulated manner.

Mutating a highly conserved tyrosine residue in phyB to a histidine (Y276H) results in a constitutively active phyB (Su and Lagarias, Plant Cell, 2007). Plants expressing phyB Y276H exhibit a *cop* (constitutively photomorphogenic) phenotype and they deetiolate in darkness. Here we show that phyA carrying the respective Y->H mutation (Y242H) interacts with FHY1/FHL in a light-independent fashion, which supports the notion that phyA Y242H is constitutively active. However, wild-type seedlings expressing phyA Y242H exhibit only a weak *cop* phenotype and they are strongly hyposensitive to far-red light - and not hypersensitive as one would expect. Co-expression of phyA-CFP and phyA Y242H-YFP shows that phyA Y242H does not properly accumulate in the nucleus and that it inhibits nuclear transport of wild-type phyA.

We present further data regarding phyA Y242H and propose a model to explain why the Y242H mutation interferes with nuclear transport of phyA.

P407 Enzymatic characterization of DAD1-like acylhydrolase families targeted to the chloroplast in Arabidopsis

Young Sam Seo1, Eun Yu Kim1, Jeong Hoe Kim2, Woo Taek Kim1

1Yonsei University, Seoul, Korea, 2Kyungpook National University, Daegu, Korea

In Arabidopsis, the DAD1-like acylhydrolase family plays important roles in fatty acid-derived signals such as jasmonate production, abiotic stress resistance, and maintaining the structural integrity of chloroplasts.

They have been divided into three classes (I-III) according to their sequence homology and the presence of signal peptide. To prove this classification experimentally, we performed gene expression studies and *in vitro* lipase assays with class I members. Real-time PCR and microscope analysis showed that seven genes belonging to class I are expressed in all tissues examined and localize to the chloroplast. Biochemical assays also revealed that all of these proteins can catalyze the hydrolysis of phosphatidylcholine at the sn-1 position. However, as discovered recently one of the chloroplast-targeting DGL protein targets galactolipid specifically for hydrolysis rather than just phospholipid. It is therefore necessary to classify class I acylhydrolases by substrate specificity in addition to sequence homology.

Therefore, we examined the enzymatic activities of seven proteins with PC, MGDG, DGDG or TAG. The results showed that these proteins are grouped into two subclasses by their substrate specificity. One group, including DGL, At2g31690, At4g16820, and At1g51440, possessed galactolipase activity, and the other consisted of DAD1, At1g06800, and At2g30550 could hydrolyze all four substrates. This indicates that each DAD1-like acylhydrolase may play a specific role in the chloroplast in spite of their close sequence homology.

P408 Iron loading during embryogenesis in Arabidopsis at tissue, cellular and sub-cellular levels revealed by *in situ* histochemical staining

Hannetz Roschzttardtz, Geneviéve Conéjéro, Catherine Curie, Stéphane Mari

Laboratoire de Biochimie et Physiologie Moleculaire des Plantes, Institut de Biologie Intégrative des Plantes, CNRS (UMR5004), INRA, Université Montpellier II, Ecole Nationale Supérieure d Agronomie, 2 Place Viala. F-34060 Montpellier CEDEX2, France.

The development of imaging techniques is of potential great interest for a better understanding of metal homeostasis in plants. The well-known Perls histochemical staining of iron is hardly usable in plants, due to its very low sensitivity. We have adapted a Fe histochemical staining in Arabidopsis embryos, based on the intensification of Perls with diaminobenzidine (DAB) in the presence of H_2O_2. Comparison of the distribution of Perls/DAB staining in embryos of wild type and *vit1* a mutant with altered Fe distribution, combined with *in vitro* tests, have established that the method, highly sensitive and straightforward, is also specific for iron in plants and detects both FeII and FeIII. The iron distribution was rather diffuse in early stages of embryo development, but iron gradually concentrated to the provascular system as it differentiated. By histological analyses of thin sections of stained embryos, we could show that Fe accumulated specifically in a single cell layer corresponding to the endodermis. In the *vit1* mutant, the diffuse Fe distribution was actually attributable to the loss of endodermal accumulation. Furthermore, in *short root* mutant embryos, lacking endodermis, Fe accumulated in the central cylinder, suggesting that endodermis functions as a barrier for the radial movement of Fe. Taken together, these data establish that the Perls/DAB method represents a powerful tool to detect iron in plant tissues, and enabled us to uncover a new role of the endodermis in iron storage in the embryo. Finally, the latest improvement of the technique enabled us to reach a sub-cellular resolution in, virtually, any kind of plant tissue.

P409 Systematic protein localization in Arabidopsis

Tao Zheng, Konstantinos Alexiou, Max Bush, Cristina Pignocchi, John Doonan

John Innes Centre, Norwich, UK

Like all eukaryotic cells, plant cells are organised into structurally and functionally distinct compartments whose organisation and composition can be highly dynamic. Moreover, the protein composition of different cellular compartments tends to be distinctive and related to the function of that compartment. Gene products can be classified into groups based on just three properties: *molecular function, biological process* and *cellular component* (subcellular localisation) (Gene Ontology Consortium, 2001). The cellular component encompasses the subcellular structures, locations and macromolecular complexes in which a protein may be found. Knowledge of the subcellular location of a gene product can be a strong indicator of the molecular function and/or the biological processes it is involved in. Therefore, as part of the EU funded Arabidopsis Growth Network integrating OMICS(AGRO-OMICS) we have set up a survey to define the subcellular localisation of proteins involved in leaf growth. As a first step, we identified a subset of growth related genes, based on the degree of co-expression with core cell cycle-related genes. An initial 115 candidates were selected from a set of genes involved or expressed during active leaf growth. Trimmed ORFs, lacking 5' and 3' regulatory sequences were transferred into entry vectors using the Gateway recombination cloning system and then moved into plant expression vectors containing an in-frame red fluorescence protein tag. The resultant vectors were transformed into Arabidopsis cells using Agrobacterium mediated transformation and the fusion proteins visualised by fluorescence microscopy. The experimental localisation data was compared with predicted localisation using a variety of databases, including SUBA (http://www.plantenergy.uwa.edu.au/applications/suba2/index.php). A number of novel localisation patterns have been described and several unknown or poorly characterised proteins have been allocated to a subcellular compartment. Examples will be discussed.

P410 Alterations in the cellular distribution pattern of AnnAt1 upon NaCl and ABA treatment

Malgorzata Lichocka, Dorota Konopka-Postupolska

Institute of Biochemistry and Biophysics, Pawinskiego 5a, 02-106 Warsaw, Poland

Plant annexins are ubiquitous and are found in different plant tissues and cell compartments. In response to abiotic stress and abscisic acid (ABA) treatment annexins have been shown to interact with negatively charged phospholipids in membranes in a calcium dependent manner. We investigated whether NaCl and ABA induce changes in the subcellular localization of Annexin 1. Our interest was specially focused on the possible association of AnnAt1 with the membrane compartments upon stress conditions. In non-treated Arabidopsis plants that overexpressed AnnAt1-GFP, the fusion proteins were mostly localized in the cell cytoplasm, associated with the nuclear and chloroplasts envelope. Because there are also evidence that *in vitro* annexins form homo-oligomers we used bimolecular fluorescence complementation (BiFC) assay to investigate whether AnnAt1 occurs *in planta* as a homo-dimers. Transient expression of BiFC vector revealed that in the plant cells AnnAt1 forms homodimers that are localized in the same cell compartments as AnnAt1-GFP fusion proteins.

Further experiments concerned subcellular distribution of AnnAt1 upon osmotic stress induced by NaCl and ABA. NaCl induces dramatic reorganization of subcellular compartments. Co-localization analyzes of AnnAt1-GFP with endoplasmic reticulum (ER) marker revealed that AnnAt1 is associated with ER membranes that undergo adaptive alterations in response to NaCl treatment. In ABA-treated plant cells we observed decrease in the vacuolar volume and formation of small vesicles. AnnAt1-GFP fluorescence was localized on the membrane of newly formed

vesicles. These results indicate that AnnAt1 might be involved in membrane reorganization induced by abiotic stress. Similar cellular localization pattern of AnnAt1 homo-dimers and AnnAt1-GFP fusion proteins suggests that in the plant cell AnnAt1 may act as a oligomeric complex. Whether AnnAt1 dimers are similarly distributed upon abiotic stress condition must be further investigated.

P411 An investigation into poly(ADP)ribosylation in *Arabidopsis thaliana*

Mai-Britt Jensen, Sarah Henry, Susan Rosser
Glasgow University, Glasgow, UK

Poly ADP ribosylation is a unique post-translational modification, characterised by the addition of complex branched poly ADP ribose polymers onto a wide variety of acceptor proteins, catalysed by the poly ADP ribose polymerase (PARP) enzymes. Poly ADP-ribose is a linear or branched polymer of many ADP-ribose units (sometimes up to 400) linked by glycosidic ribose-ribose bonds. The reaction utilises NAD+ as the substrate and the activity of the PARP enzymes is directly linked to the concentration of NAD+ in cells. The removal of polymers is carried out by the catalytic actions of the enzyme poly(ADP)ribose glycohydrolase (PARG), which ensures rapid turnover of the polymer in the cell. There is an ongoing interest in PARP and its activity due to its association with the immediate cellular response to DNA damage and in particular with the DNA strand break repair machinery. In *Arabidopsis thaliana* there are 3 PARPs, two of which have been shown to be upregulated upon exposure to genotoxic stress, and a third uncharacterised PARP.

We have identified Arabidopsis plant lines mutated in the putative *PARP* and *PARG* genes and investigated their tolerance to a range of abiotic stresses, in particular genotoxic stress. *Arabidopsis thaliana* has two putative *PARG* genes and a single basepair mutation in the highly conserved catalytic region of *AtPARG-1* has previously been shown to affect the circadian clock resulting in a lengthening of period which manifested as an early flowering phenotype (Panda *et al*, 2002). The putative Arabidopsis *PARG* genes have been cloned and expressed in hetrologous hosts, the resulting proteins purified and demonstrated to have poly(ADP)ribose glycohydrolase activity *in vitro*. Unlike other organisms the insertional inactivation of the PARG enzymes in Arabidopsis does not result in embryo lethality potentially providing a very valuable system to identify novel substrates for PARP enzymes.

P412 Artificial microRNA specifically down-regulates cysteine synthesis in mitochondria

Florian H Haas, Corinna Heeg, Markus Wirtz, Rudiger Hell
University of Heidelberg, Heidelberg, Germany

Cysteine synthesis is catalyzed by serine acetyltransferase (SAT) and O-acetylserine(thiol)lyase with O-acetylserine (OAS) as intermediate. Why this pathway occurs in cytosol, plastids and mitochondria and if the sulfur-related metabolites are exchanged remains unsolved. Mitochondrial SAT activity is high but OAS-TL activity is low compared to the rest of the cell in *Arabidopsis thaliana*. To investigate the role of mitochondria in cysteine synthesis down-regulation of *Sat3*, the gene encoding mitochondrial SAT3, was achieved using an artificial micro RNA approach.

Sat3 expression was significantly knocked-down in a series of independent T-DNA transformants expressing *amiSat3* RNA and resulted in severe dwarfism as strongest phenotypes in the T1 generation. These growth retardations were inherited in the T2 generation, although at a reduced extent with strongest affected T1 lines showing relatively largest release from dwarfism. The phenotypical growth reduction in size of the plants strictly correlated with the reduction of *Sat3* mRNA, SAT3 protein level and SAT total enzymatic activity in leaves. SAT activity in Arabidopsis is encoded by a family of five genes. The specificity of down-regulated *Sat3* mRNA was shown by three independent experiments: semi-quantitative RT-PCR, quantitative real-time PCR and microarrays using different gene-specific oligonucleotides as primers and for hybridization, respectively. None of the other *SAT* genes was affected by *amiSat3* RNA. The levels of OAS in plants expressing the *amiSat3* RNA were strongly reduced by 80% and, most importantly, the flux of 3H-labeled serine into cysteine was down by 50% compared to wild type plants of the same age. The results show that amiRNA can be used to specifically down-regulate individual members of a gene family and mitochondrial SAT3 is the rate-limiting step in OAS formation and thus overall cellular cysteine biosynthesis.

P413 Spatial regulation of ROP4 and GDI1 in root hair growth

Xin Chen, Claire S Grierson
University of Bristol, Bristol, UK

Root hairs are long, thin tubular outgrowths of epidermal cells on roots. Root hair cells provide a model system for studying cell polarity and polar growth. ROPs, the plant specific family of Rho small GTPases, are believed to function as molecular switches in regulating root hair growth. ROP2 positively regulates root hair development. GFP::ROP2 localizes to the future site of hair formation even before swelling formation and to the tip throughout hair development (Jones, *et al*, 2002). GDI1, a negative ROP regulator, is also expressed in root hairs and has a strong root hair phenotype (Carol, *et al*, 2005). The loss-of-function mutant *gdi1* (also known as *scn1-1*) has a very similar phenotype to lines in which ROP2 is over-expressed. The root hair phenotype of a loss-of-function *rop4* T-DNA insertion line and microarray data both suggest that ROP4, which has 97% similarity at the amino acid level to ROP2, is also involved in root hair development. Laser scanning confocal microscopy to explore the extent of sub-cellular co-localization of YFP::ROP4 and CFP::GDI1 in developing root hairs, together with interaction studies of ROP4 and GDI1 will be presented.

P414 Functional analysis of GONST3 and 4, nucleotide-sugar transporters of *Arabidopsis thaliana*

Michael Handford, José Patricio Miranda, Diego Ampuero, Mariela Huichalaf, Sebastian Febres
Universidad de Chile, Santiago, Chile

Specific transporter proteins are required for the import of cytosolically-synthesised nucleotide-sugars into the lumen of the Golgi apparatus for the synthesis and/or modification of glycoproteins and non-cellulosic polysaccharides. Using a reverse genetics approach, we have identified GONST3 and 4 from *Arabidopsis thaliana*. Both possess the molecular characteristics of previously-identified nucleotide-sugar transporters (NSTs) which transport GDP-sugars, but not UDP-sugars. Phylogenetic analysis suggests that GONST3 and GONST4 arose early in the evolution of NSTs. Our work is focussed on determining their substrate specificity and on analysing their role *in planta*. To achieve these aims, GONST3 and 4 were fused to epitope tags; GONST4-His and GONST4-GFP were localised to the Golgi apparatus in agro-infiltrated tobacco leaves.

GDP-L-galactose, which is not available commercially, is a potential substrate of GONST3 and 4. We synthesised GDP-L-galactose enzymatically from GDP-D-mannose for use in transport assays. Both GONST4-His and GONST4-GFP were capable of transporting GDP-L-galactose and GDP-L-fucose, but not GDP-D-mannose or UDP-D-glucose, into the lumen of a Golgi-enriched fraction extracted from agro-infiltrated tobacco leaves. Similar experiments with GONST3 are underway.

In addition, to determine their function *in vivo*, GONST3 and 4 expression levels were reduced by post-transcriptional gene silencing. Using Arabidopsis lines transformed with promoter-GUS fusion constructs, both NSTs are highly expressed in specific floral organs and roots and exhibit differential expression profiles during early stages of development. Advances in the analysis of the morphology and composition of L-fucose- and L-galactose-containing glycoproteins and polysaccharides will be shown. GONST4 is thus the first known NST capable of transporting GDP-L-galactose and the only known polysaccharide requiring this substrate is the pectin, rhamnogalacturonan II.

Funding: Fondecyt Iniciación 11060470

P415 AtSDL, a putative sugar-alcohol dehydrogenase in *Arabidopsis thaliana*

Patricio Mandujano, Daniel Severin, Marìa Francisca Aguayo, Michael Handford
Universidad de Chile, Santiago, Chile

Sugar alcohols, including sorbitol, mannitol and xylitol, perform a variety of roles in plants, including as a means of transporting carbon long-distance in the phloem and as compatible solutes. The synthesis and breakdown of these compounds has been studied extensively, and several NAD-dependent sorbitol dehydrogenase (*SDH*) genes have been cloned and characterised from plants. Although sorbitol is the main substrate of these SDHs, other sugar alcohols are also oxidised. In *Arabidopsis thaliana*, a species which possesses low basal levels of several sugar alcohols, we have identified a putative SDH (AtSDL). AtSDL shares >75% amino acid identity with known plant SDHs and structurally and catalytically important residues are conserved. Our aim is to biochemically characterise this enzyme.

RT-PCR assays demonstrate that *AtSDL* is expressed in multiple plant organs, including leaves, roots, stems, seeds and flowers. In silico analyses of *AtSDL* expression during development and under different growth conditions are currently underway. Using Arabidopsis lines transformed with an AtSDL promoter::GUS fusion construct, a more-detailed expression profile has been determined, showing for example that expression in flowers is restricted to sepals and filaments. Transient expression of AtSDL-GFP in tobacco leaves indicates that the protein is localised in the cytosol, consistent with the subcellular localisation of previously-described SDHs and with in silico analysis of the protein sequence.

In order to determine the substrate specificity of the enzyme, recombinant AtSDL-His was overexpressed in Escherichia coli. After purification and under denaturing conditions, AtSDL-His was capable of oxidising sorbitol, and other substrates are currently being examined. To this end, three-dimensional modelling and molecular docking studies of the protein indicate that NAD is an acceptable cofactor, a finding which was confirmed experimentally.

P416 The *MUM1* gene is required for seed coat mucilage extrusion in Arabidopsis

Jun Huang1, Gillian Dean1, Jonathan Griffiths1, John Gotz2, Shawn Mansfield1, George Haughn1
1University of British Columbia, Vancouver BC, Canada, 2University of Melbourne, Melbourne, VIC, Australia

During differentiation, the Arabidopsis seed coat epidermal cells produce copious amounts of mucilage that is extruded from the seed coat upon imbibition. Mucilage is composed primarily of pectin, a polysaccharide that is a main component of the cell wall. For this reason, the Arabidopsis seed coat is a good system for studying the biosynthesis, secretion and/or modification of pectin. Mutants with mucilage defects can be used to identify genes involved in the biosynthesis and modification of pectin. Two such Mucilage-Modified mutants, *mum1* and *mum2*, were identified by our screens of EMS mutagenized plants. Both lack the ability to release the mucilage when mature seeds are imbibed. The structure and development of the seed coat epidermal cells of both mutants showed no difference from that of wild type. Cloning of *MUM2* revealed that it encodes a beta-galactosidase that modifies the mucilage structure in the apoplast. We have recently cloned the *MUM1* gene and shown it to encode a putative transcription factor with WD40 repeats at C terminus. Cellular localization and transcriptional assay results indicate that MUM1 is a nuclear-localized, transcriptional activator. *MUM1* is expressed in all the tissues examined including the seed coat. qRT PCR data suggests that *MUM1* is expressed throughout seed coat development, reaching peak expression late in

differentiation. *MUM2* expression in the *mum1* mutant decreased dramatically, compared to that of wild type. Overexpression of *MUM1* could partially rescue the *mum2* phenotype. These data suggest that MUM1 is a positive regulator of *MUM2*. qRT PCR data from seed coats revealed a similar expression level of *MUM1* in wild type compared to plants homozygous for mutations in several genes encoding regulators of seed coat mucilage, namely *APETALA2, TRANSPARENT TESTA GLABRA1 (TTG1), TTG2* and *GLABRA2*. Thus the *MUM1-MUM2* regulatory pathway appears to be independent of other transcription factors known to regulate aspects of seed coat mucilage biology.

P417 AKR2A-mediated import of chloroplast outer membrane proteins is essential for chloroplast biogenesis

Dae Heon Kim, Wonsil Bae, Yong Jik Lee, Yoon Jung Na, Zhengyi Xu, Inhwan Hwang

Division of Molecular and Life Sciences, Pohang University of Science and Technology, Pohang, Korea

In plant cells, chloroplasts play essential roles in many biochemical reactions and physiological responses. Chloroplasts require numerous protein components, but only a fraction of these proteins are encoded by the chloroplast genome. Instead, most are encoded by the nuclear genome and imported into chloroplasts from the cytoplasm post-translationally. Membrane proteins located in the chloroplast outer envelope membrane (OEM) play a critical role in the import of proteins into the chloroplast. However, the biogenesis of chloroplast OEM proteins remains poorly understood. Here, we report that an Arabidopsis ankyrin repeat protein, AKR2A, plays an essential role in the biogenesis of the chloroplast OEM proteins. AKR2A binds to chloroplast OEM protein targeting signals as well as to chloroplasts. It also displays chaperone activity towards chloroplast OEM proteins, and facilitates the targeting of OEP7 to chloroplasts *in vitro*. *AKR2A* RNAi in plants having an *akr2b* knock-out background showed greatly reduced levels of chloroplast proteins, including OEM proteins, and defective chloroplast biogenesis. Thus, AKR2A functions as a cytosolic mediator for sorting and targeting of nascent chloroplast OEM proteins to the chloroplast.

P418 Investigating the essential role of dynamin in Arabidopsis growth and development

Neil Taylor

University of York, York, UK

Endocytic mechanisms control the lipid and protein content of the plasma membrane, thereby regulating the way in which cells interact with one another and with their environments. In plants, endocytosis is important in a number of processes, including recovery of excess plasma membrane during tip growth, internalisation of receptor like kinases and recycling of proteins, including PIN proteins involved in polar auxin transport. Despite the importance of endocytosis in plants, details of its mechanism and regulation remain poorly understood.

In animal systems, dynamin is required for numerous endocytic processes. Dynamins are large GTPases which assemble to form spirals around the neck of invaginating vesicles followed by GTPase dependant scission of the vesicle to release it from the plasma membrane by a squeezing, twisting or stretching action.

The Arabidopsis genome encodes two homologs of mammalian dynamin (*DRP2A* and *DRP2B*) that contain all the domains found in animal dynamins. The encoded proteins are 92% identical, and both genes are expressed ubiquitously. T-DNA knockouts of either gene do not show any apparent phenotype. Double knockouts cannot be recovered however, and reciprocal crosses have shown that a total lack of dynamin is gamete lethal. A dominant negative approach has therefore been taken to study the role of dynamin in plants. Expression of a GTPase defective dynamin has been shown in other systems to interfere with wild-type dynamin function. Constitutive expression of a GTPase defective dynamin in plants allows only the recovery of transgenic lines expressing very low levels of the mutated protein compared to control constructs expressing the wild-type protein, indicating that the GTPase defective dynamin is acting in a dominant negative manner. Plants carrying the GTPase defective dynamin under the control of an inducible promoter have therefore been developed. Upon induction these plants can accumulate mutated protein at levels several fold that of the endogenous wild-type protein and are currently being used to study the role of dynamin in a number of cell types. An understanding of the role of dynamin in endocytosis will enable us to further dissect the way in which this essential process is regulated.

P419 Seed storage protein trafficking and localization in leaves of an Arabidopsis LEAFY COTYLEDON2 over-expression line

M Feeney1, Y Cui1,2, J J Harada3, R Menassa1,2

1 Department of Biology, University of Western Ontario, London, ON, Canada, N6A 5B7 2 Agriculture and Agri-Food Canada, London, ON, Canada, N5V 4T3, 3 Section of Plant Biology, Division of Biological Sciences, University of California, Davis, CA 95616, USA

Seed storage proteins (SSPs) are typically synthesized exclusively in seeds and are then stored within protein storage vacuoles (PSVs). In vegetative tissues such as leaves, it is unclear whether PSVs are present. This research aims to discover whether PSVs can exist in leaves and whether they accumulate SSPs. To achieve this, one of the most abundantly expressed SSP families in Arabidopsis, the 2S albumin proteins, is being studied and the organelle in which the proteins accumulate will be identified. Several Arabidopsis mutants and over-expression lines that express embryogenic characteristics in vegetative tissues were evaluated for the presence of 2S albumin proteins in their leaves. The 2S albumins were detected in the LEAFY COTYLEDON2 (LEC2) over-expression line by immunoblotting. The presence of a storage organelle, which accumulates the seed proteins, will be identified by histochemical staining. To localize SSPs and PSVs at a subcellular level, translational gene fusions will be transiently expressed in LEC2. The translational

fusions will consist of a fluorescent reporter linked to either a SSP or to organelle markers. Using confocal microscopy, the subcellular localization of SSPs in leaves will be detected and accumulation of these proteins will be co-localized with the storage organelle. Finally, the developmental pattern of SSP expression and the presence of the storage organelle in LEC2 will be studied. This research will further our understanding of protein trafficking and storage in leaves and may have valuable applications for improving recombinant protein accumulation in plants.

P420 RETINOBLASTOMA-RELATED protein (RBR) integrates regulation of cell cycle and acquisition of cell polarity in *A. thaliana*

Lorenzo Borghi, Ruben Gutzat, Johannes Fütterer, Wilhelm Gruissem

Institute of Plant Sciences, ETH Zürich, Switzerland

Several genes and hormones involved in the control of post-embryonic organ initiation and growth have been identified. However, it remains unclear how these differentiation cues connect to genes that regulate the cell cycle. With an inducible RNAi system (1) against Arabidopsis *RBR*, a key regulator of the cell cycle, we perturbed *RBR* expression levels at different stages of plant development. We report here that conditional reduction of *RBR* expression not only promoted context-dependent cell proliferation, but also negatively influenced the establishment of cell polarity. Additionally, meristem activity, auxin distribution, and lateral organ formation and development were strongly altered. The effects of RBR pathway perturbation establish RBR as a master regulator of the cell cycle and a key factor for synchronizing cell division and cell differentiation. Our results indicate a role of RBR in establishing cell polarity.

1 Plant J. 2005;41(6):899-918

Systems Biology

P421 Reconstructed Arabidopsis transcriptional network identified key genes in stress tolerance

Qing Zhang1, Pui-Man Yu2, Dianjing Guo1

1The Chinese University of Hong Kong, Hong Kong SAR, China, 2The Chinese University of Hong Kong, Hong Kong SAR, China

Regulation of gene expression is one of the most important processes in cellular systems. It is believed that gene expression is largely controlled at transcription level, where the interaction between regulatory transcription factors (TFs) and the target genes play a crucial role. Plants respond to various internal and external cues via interconnecting networks of multiple genes and regulatory proteins. Unraveling such networks is of utmost importance in deciphering how genes and systems function in stress responses.

We present a modified probabilistic graphical framework for transcriptional network inference. By applying our algorithm to published gene expression data in *Arabidopsis thaliana*, we reconstructed a transcription network underlying stress responses and identified hub genes involve in stress responses. We verified the computationally predicted hub gene using transgenics and RT-PCR approach. Transgenic Arabidopsis plants overexpressing the identified MYB gene showed enhanced tolerance to salt treatment.

P422 Trehalose-6-phosphate and sugar signalling in plants

Umesh Yadav, Regina Feil, Mark Stitt, John Lunn

Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg1, 14424 Potsdam, Germany

Trehalose-6-phosphate (Tre6P) is an essential signal metabolite that regulates carbohydrate metabolism and development. Mutants with altered Tre6P levels show drastic changes in their phenotypes and metabolism, however, the precise function of Tre6P in plants is still unclear. It has been proposed that Tre6P acts as a signal of sucrose status in plants (Lunn *et al*, 2006). We are investigating the mechanistic details of how Tre6P linked to the level of sucrose. Tre6P is synthesized from UDP-glucose and glucose-6-phosphate by trehalose-phosphate synthase (TPS), and dephosphorylated to trehalose by trehalose-phosphatase (TPP).

The *Arabidopsis thaliana* genome contains a family of eleven genes (*AtTPS1-11*) encoding TPS or TPS-like proteins, and a family of ten genes (*AtTPPA-J*) encoding TPP. Microarray and Real-Time RT-PCR analyses showed dramatic changes in the abundance of transcripts from several TPS genes in response to altered sucrose levels. Only *AtTPS1* and *AtTPS6* have been reported to encode active TPS enzymes, however, sucrose has little or no effect on the transcript levels of these two genes. The sucrose-induced increase in Tre6P is blocked by cycloheximide (a translational inhibitor), but cordycepin (a transcriptional inhibitor) had little effect, suggesting that protein synthesis, but not *de novo* transcription, is essential for the response. However, ribosome-loading experiments indicated that trehalose metabolic genes are not regulated at the level of translation, suggesting that synthesis of some other proteins is required for the Tre6P response to sucrose. Inhibition of the 26S proteasomal protease by MG-132 enhanced the sucrose-induced rise in Tre6P.

Together, these observations indicate that a short-lived protein, which is either an activator of TPS or an inhibitor of TPP, could be necessary for the Tre6P reponse to sucrose.

P423 A strategy for inferring gene regulatory networks from time series transcriptomics data

Kim Kenobi, Andy Wood

University of Nottingham, Nottingham, UK

In the Centre for Plant Integrative Biology in Nottingham, one of the questions we are interested in is how to infer the gene regulatory network of Arabidopsis roots exposed to gibberellin (GA). Our data set will consist of four replicates at each of 9 time points following exposure of the

roots to GA. We are interested in different zones within the root, so the experiment is carried out on five different zones.

One of the challenges in inferring the gene regulatory network from such a data set is that the number of genes (approx. 22000) is a lot more than the number of microarrays (36 for each of the five zones).

In order to tackle this problem we propose a technique that models the rate of change of (log) mRNA expression levels for a particular gene at a particular time as a linear combination of the mean (log) expression levels of all of the genes in the transcriptome at that time. The problem thus reduces to a linear regression problem in which we want a large number of the regression coefficients to be equal to zero. There is a type of linear regression called "lasso regression" that does exactly this.

In this poster, we outline the challenges in applying lasso regression to the problem of inferring gene regulatory networks. We illustrate our work with Arabidopsis time series transcriptomics data sets, and present a set of genes that are stable across changes in the parameters of the model and changes in the selection criteria for the lasso regression. We also explore the problems associated with the sparsity of the gene ontology for Arabidopsis and the lack of "gold standards" against which to assess the efficacy of our network inference algorithms.

P424 Using expression correlation and reaction correlation data for predicting enzyme localisation in multi-compartmental systems

Achuthanunni Chokkathukalam, Mark Poolman, Lee Sweetlove, David Fell

Oxford Brookes University, School of Life Sciences, Oxford, UK

Compartmentation of enzymes and other proteins is one of the distinguishing characteristics of plant cells. Although technologies such as MS and GFP have accelerated the flow of enzyme and protein localisation information, the subcellular location of majority of proteins in a plant cell is still unknown. Software packages providing localisation data are often inconsistant with each other, or with experimental results.[1]

We describe a method by which localisation of enzymes in a plant cell may be predicted using transcription profiles combined with the structure of the metabolic network. The method uses reaction correlation coefficient (RCC)[2] to identify reactions that carry similar flux. Once a transcription correlation matrix for the genes of interest is calculated, the columns are clustered using the expression correlation coefficient and the rows clustered using RCC. In the resulting matrix, we show that the genes targeting a particular compartment are clustered together and compartmental predictions, with respect to a reference gene can be readily made.

The Python based metabolic modelling tool ScrumPy[3] was used to construct a model of plant metabolism containing light reactions, Calvin cycle, glycolysis and the TCA cycle. Genes coding for enzymes catalysing reactions in the model were extracted from AraCyc. Expression values were obtained from the NASC array data and segregated using a manually annotated set of experiments (Morandini, Uni. of Milan, unpublished).

Predicted enzyme locations are compared to those made by existing software. To further confirm the accuracy of this approach, 22 genes were selected and subjected to GFP analysis, the results of which will be presented.

1 Lunn, J.E., Compartmentation in plant metabolism, J. Exp. Bot., 58, 35-47, 2007
2 Poolman *et al*, Modular decomposition of metabolic systems via null-space analysis. J. Theor. Biol., 249, 691-705, 2007
3 Poolman *et al*, ScrumPy - Metabolic modelling with Python. IEE Proc. Sys. Bio., 153, 375-378, 2006

P425 Network inference to identify regulators of Arabidopsis leaf senescence

Vicky Buchanan-Wollaston, Emily Breeze, Stuart McHattie, Andrew Mead, David Wild

University of Warwick, Warwick, UK

Leaf senescence is a programmed event responding to a wide range of external and internal signals including those caused by development, age and environment. Senescence requires *de novo* gene expression and protein synthesis and is controlled in a tightly regulated manner. Identification of the genes that control senescence has been complicated by the complex combination of signalling pathways that appear to be involved in senescence. Cross talk exists between senescence and stress or pathogen responses and also the hormonal and nutrient signals that are implicated in the control of senescence.

We are using Arabidopsis as a model, taking a systems biology approach, to identify the genes involved with the control of leaf senescence. Extensive high resolution timecourse microarray analysis has been analysed using a variational Bayesian State Space modelling method and transcriptional networks that pinpoint key regulatory genes operating to control gene expression during developmental leaf senescence have been generated. Mutant analysis with some of the potential hub genes has shown that several of these are important for the normal senescence process. We are using various clustering techniques together with promoter motif analysis to characterise the global changes in gene expression during senescence. This analysis is being used to group potentially co regulated genes. In addition, cross talk between stress related pathways and senescence is being elucidated by the use of mutants, stress treatments and comparative gene expression analysis.

P426 In-depth profiling of leaf growth

Pierre Hilson, the AGRON-OMICS Consortium

Dept. Plant Systems Biology

The circuitry that drives growth at the different levels of organisation (whole plant, organ, cell, molecular module, molecule) largely remains to be established. In

that framework, we have undertaken the exhaustive profiling of a developing leaf to further characterize the molecular mechanisms underpinning plant organ growth.

The reference in this experimental series is the sixth rosette leaf of the Columbia ecotype grown in optimal conditions. Pooled leaf samples were harvested after primordium formation at four successive stages of development corresponding to active cell division, rapid cell expansion, end of expansion and maturation, by the end of day and night. The biological samples are being analyzed for macroscopic and microscopic growth parameters, DNA content, cell wall polymer composition, and are profiled for metabolites, lipids, primary metabolism enzymatic activities, transcripts, proteins and epigenetic marks.

The resulting integrated dataset will provide a unique baseline to investigate the consequences of genetic and environmental perturbations on the leaf system.

P427 Extended computational model of the circadian clock genetic network in Arabidopsis

Alexandra Pokhilko, Kirsten Knox, Kieron D Edwards, Adrian Thomson, Andrew J Millar

Edinburgh University, School of Biological Sciences

Daily changes in plant rhythms are driven by cyclic expression of the circadian clock genes. Our previous computational model of the clock genetic network consists of three feedback loops, which represent connected morning and evening oscillators (Locke *et al* Mol. Syst. Biol. 2: 59, 2006). Here we extend the model based on recently-published data, make new predictions and test them experimentally.

The morning loop of auto-regulation of *LHY/CCA1* genes by the inhibitors PRR9/PRR7 was extended by adding a proposed clock component, the Night Inhibitor, which might combine the functions of CHE and PRR5 (Pruneda-Paz *et al* Science 323: 1481, 2009; Nakamichie et al Plant Cell Physiol. 46: 609, 2005). The evening loop was modified to include the post-translational regulation of TOC1 by the F-box protein ZTL, which is stabilized by GI protein (Kim *et al* Nature 449: 356, 2007). GI's role in the clock model has thus been revised according to published data: GI promotes inhibition of TOC1 protein through positive regulation of ZTL.

A new hypothesis for TOC1 function has modified the connection between evening and morning loops, accounting for additional data that contradicted earlier models. We used the model to predict the clock's response to various perturbations, such as changes in light conditions or mutations of the clock genes, and have tested these predictions experimentally.

Our results show that the rhythmic amplitude and phase of the key clock genes *LHY* and *CCA1* are determined by the combined functions of the Night Inhibitor and the activator TOC1.

P428 What are the downstream targets of trehalose 6-phosphate signalling in plants?

Marina C M Martins1, Jörg Fettke2, Regina Feil1, Mark Stitt1, John E Lunn1

1Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany, 2University of Potsdam, Institute of Biochemistry and Biology, Potsdam-Golm, Germany

There is now considerable genetic and biochemical evidence that trehalose 6-phosphate (Tre6P), the intermediate of trehalose biosynthesis, is an essential signal metabolite in plants. It appears to be a signal of sugar status, and clearly plays a major role in the control of plant metabolism and development, although the mechanism of action of this sugar remains unclear. The main aim of this project is to discover the signal transduction pathways that link Tre6P to its downstream targets. *Arabidopsis thaliana* plants have been transformed with constructs for ethanol-inducible expression of the enzymes that synthesize and hydrolyse Tre6P trehalose-phosphate synthase (TPS) and trehalose phosphatase (TPP). Several metabolites from glycolysis, TCA cycle and photosynthesis were measured, using LC-MS, HPLC and enzymatic assays. High Tre6P levels leads to some changes in TCA cycle intermediates and amino acids in the end of the day and end of the night. The most surprising result was a pronounced inhibition of starch breakdown in leaves containing high levels of Tre6P at night. Microarray analysis showed that Tre6P affects only a small subset of sucrose-responsive gene transcripts. However, several genes do respond specifically to increased Tre6P levels, and these are potential reporter genes for a screen to identify Tre6P-signalling mutants. With knowledge of the downstream targets and intermediates of Tre6P signalling, it should be possible to incorporate this pathway into integrated models of the regulatory networks that control plant metabolism and development.

P429 The design principle of robust disease resistance

Kenichi Tsuda1, Masanao Sato1,2, Jane Glazebrook1, Fumiaki Katagiri1

1Dept of Plant Biol, Microbial and Plant Genomics Inst, Univ of Minnesota, St Paul, MN, USA, 2Dept of Life Sci, Grad Sch of Arts and Sci, Univ of Tokyo, Tokyo, Japan

Effector-triggered immunity (ETI) is a form of plant inducible defense against pathogens. It is robust against pathogenic or genetic perturbations to its signaling mechanism. To test the possibility that this robustness is achieved by functional redundancy, we constructed an Arabidopsis quadruple mutant *dde2/ein2/pad4/sid2*. DDE2, EIN2, PAD4, and SID2 define the signaling sectors mediated by jasmonate, ethylene, salicylate/unknown, and salicylate, respectively. The level of ETI was measured by comparing the growth of *Pseudomonas syringae* pv. *tomato* DC3000 (Pto DC3000) expressing the effector AvrRpt2 to the growth of Pto DC3000 carrying the empty vector. In the quadruple mutant, 80% of AvrRpt2-ETI was lost, i.e., the signalling network defined by the four genes accounts for most of AvrRpt2-ETI. To precisely estimate the effects of single wild-type genes and their interactions, we measured AvrRpt2-ETI in plants carrying

all possible combinations of the four mutations and fitted a mixed general linear model to the obtained data. This signalling allocation analysis demonstrated that each of the four genes contributes positively to resistance and that functional redundancy among the four genes is the basis of robust AvrRpt2-ETI. We also investigated the relationships among expression profiles in 21 Arabidopsis single defense mutants during Pto DC3000 AvrRpt2 infection using a recursive non-linear dimensionality reduction method to infer the topology of the signalling network defined by the 21 genes. This analysis revealed that negative regulation among different signalling sectors is very common, which explains the functional redundancy seen in the signalling allocation analysis. Thus, robust AvrRpt2-ETI is comprised of positively contributing signalling sectors and negative regulation among them.

P430 Simple models of circadian oscillations identify signalling network architecture

Neil Dalchau, Katharine Hubbard, Carlos Hotta, Fiona Robertson, Helen Briggs, Guy-Stan Bart, Jorge Gonçalves, Alex Webb

University of Cambridge, UK

The circadian clock is a major regulator of cellular activities, including changes in the concentration of the concentration of free Ca^{2+} in the cytosol ($[Ca^{2+}]_{cyt}$). These circadian oscillations of $[Ca^{2+}]_{cyt}$ are also regulated by the light-dark cycle. We have reconstructed the cellular network controlling cycles of $[Ca^{2+}]_{cyt}$ in Arabidopsis using reverse engineering and reverse genetics. A simple linear second order model derived from experimentally obtained input-output data captures the complex nonlinear behaviours, describes the major connections and filters in the signalling network, and identifies time-dependent processes. We demonstrate that the oscillation is responsive to different wavelengths of light in the morning compared with the afternoon. Using a similar approach we also modelled the network controlling the activity of the *CHLOROPHYLL A/B BINDING PROTEIN 2 (CAB2*) promoter to allow quantitative comparison of the two oscillations. This revealed that broadly similar rhythmic behaviours are regulated by networks with different system architecture, suggesting either important functional differences or that selection pressure is at the level of the dynamic response.

P431 ^{15}N metabolic labelling as a tool to study stress-induced dynamic changes in plasma membrane protein composition in Arabidopsis

Waltraud Schulze, Sylwia Kierszniowska, Wolfgang Engelsberger, Dirk Walther

Max Planck Institut for molecular Plant Physiology

Stable isotope labelling of plant tissues with ^{15}N has become a widely used tool in quantitative plant proteomics. We developed a robust experimental design and data analysis workflow implementing two biological replicates with reciprocal labelling and a series of 1:1 control mixtures. Thereby, we are able to unambiguously distinguish (i) inherent biological variation between cultures and (ii) specific responses to a biological treatment. The data analysis workflow is based on first determining the inherent variation between cultures based on ^{15}N to ^{14}N ratios in independent 1:1 mixtures before a biological treatment is applied. In a second step, ratio-dependent standard deviations are applied to the set of reciprocally labelled biological experiments. This allows us to define those proteins showing significant biological response superimposed on the biological variation before treatment.

Using the above described ^{15}N metabolic labeling approach, we analyzed changes in composition of plant plasma membrane in response to adaptations to changing nutrient environment. Plants need to sense their nutrient status, and adapt the composition of the plasma membrane accordingly (transport, metabolism, etc). We use established starvation-resupply experiments to analyze immediate short-term responses when plant cells experience nutrient resupply after starvation of the macronutrients carbon, nitrogen and phosphorous.

In total 44 different proteins were identified as being recruited to the plasma membrane after phosphate resupply, while 34 proteins were depleted from the membrane within 30 minutes. Especially GPI-anchored proteins, proteins with functions in cell wall were recruited to the membrane, while proteins with transport functions and in vesicle trafficking were depleted. In combination with the analysis of fast phosphorylation responses after nutrient resupply, we gain novel insights into adaptive responses in plant cells when external nutrient conditions are changing.

P432 Identification of the enzymes catalysing the final reactions of purine degradation in *Arabidopsis thaliana* and *Escherichia coli* using comparative genomics

Andrea K Werner, Claus-Peter Witte

Freie Universitaet Berlin, Germany

The availability of whole genome sequences boosts the identification of biochemical pathways conserved across species employing tools of comparative genomics. A cross-organism protein association analysis allowed us to identify two enzymes, ureidoglycine aminohydrolase and ureidoglycolate amidohydrolase, that catalyse the final reactions of purine degradation in *Arabidopsis thaliana*. A similar pathway was found in *Escherichia coli* while an alternative metabolic route via ureidoglycine transaminase can be predicted for other organisms. The four final steps of Arabidopsis purine degradation occur in the endoplasmic reticulum and do not require urease for full release of the purine ring nitrogens as ammonia.

P433 Integrative analyses of genetic variation in primary carbohydrate metabolism reveal distinct modes of regulation in *Arabidopsis thaliana*

Joost J B Keurentjes1, Ronan Sulpice2

1Laboratory of Genetics and Plant Physiology, Wageningen University, The Netherlands, 2Max Planck Institute for Molecular Plant Physiology, Golm, Germany

Plant primary carbohydrate metabolism is complex and flexible, and is regulated at many levels. Changes of

transcript levels do not always lead to changes in enzyme activities, and these do not always affect metabolite levels and fluxes. To analyze interactions between these three levels of function, we have performed parallel genetic analyses of 15 enzyme activities involved in primary carbohydrate metabolism, transcript levels for their encoding structural genes, and a set of relevant metabolites. Quantitative analyses of each trait were performed in the *Arabidopsis thaliana* Ler x Cvi recombinant inbred line (RIL) population and subjected to correlation and quantitative trait locus (QTL) analysis (Keurentjes *et al*, 2008).

Traits affecting primary metabolism were often correlated, possibly due to developmental control affecting multiple genes, enzymes, or metabolites. Moreover, the activity QTLs of several enzymes co-localized with the expression QTLs (eQTLs) of their structural genes, or with metabolite accumulation QTLs of their substrates or products. In addition, many trait-specific QTLs were identified, revealing that there is also specific regulation of individual metabolic traits. Regulation of enzyme activities often occurred through multiple loci, involving both *cis*- and *trans*-acting transcriptional or post-transcriptional control of structural genes, as well as independently of the structural genes.

These results show that multiparallel QTL analyses of the various interconnected transducers of biological information flow can assist in determining the cause and consequences of genetic regulation at different levels of complex biological systems.

Keurentjes, J.J.B., Sulpice, R., Gibon, Y., Steinhauser, M.C., Fu, J., Koornneef, M., Stitt, M., and Vreugdenhil, D. (2008). Integrative analyses of genetic variation in enzyme activities of primary carbohydrate metabolism reveal distinct modes of regulation in *Arabidopsis thaliana*. Genome Biol 9, R129.

P434 A novel centrality framework for causal gene regulatory network reverse engineering

Federico M Giorgi1, Tony Bolger, Marek Mutwil1, Zoran Nikoloski2, Staffan Persson1, Bjoern Usadel1

1Max Planck Institute for Molecular Plant Physiology, Golm, Germany, 2Institute of Biochemistry and Biology, University of Potsdam, Germany

Genome-scale gene co-expression networks have been widely used to predict gene-gene interactions, as well as to identify biological pathways and infer gene function using a “guilt-by-association” approach. There are many “retro-engineering” methods available to infer large-scale gene regulatory networks from high-throughput gene expression experiments.

These can be divided into “direct” methods, that detect a pure co-behavior of gene pairs, and “conditional” methods, which can remove indirect connections between genes upon conditioning on a common variable. In general, conditional methods seem to be better suited to detect the real underlying causal network.

Combining linear and non-linear conditional methods in a gene-oriented way, we developed a derived measure called “B-potential” and showed that in *A. thaliana* essential genes have a higher B-potential than non-essential ones. We propose this B-potential as a novel marker for genetic centrality, which doesn’t merely consider the connectivity of a gene, but possibly the amount of true “information” vehiculated by it. We will also show that B-potential is not just a by-product of network degree, but it confers an additional predictive power for gene lethality.

Extending this method to weakly expressed genes, we propose a method to take into consideration weak but locally significant correlations among genes, in order to put transcription factors “back into the game”. Our method then over-weights genes that have a high B-potential. Our framework potentially provides not only a more sensitive technique for causal gene network inference, but also a novel *in silico* marker for gene lethality. Furthermore, combining network inference, B-potential and sequence/domain analysis, it will be possible to detect new candidate transcription factors within their biological context.

P435 Using a model of the transcript response of *Arabidopsis thaliana* as a predictor for important factors during day-night cycles

Federico M Giorgi, Armin Schlereth, Wolf-Ruediger Scheible, Mark Stitt, Bjoern Usadel

Max Planck Institute for Molecular Plant Physiology, Golm, Germany

Plants as sessile organisms have to develop intricate response mechanisms to adapt to different availabilities of nutrients and light. We have previously shown that gene expression during a diurnal cycle in wild type plants can be modeled to some extent by additive linear models (Usadel *et al* 2008). In this knowledge-driven model we simply predicted the global transcript response based on the response to light, carbon and the circadian cycle, which probably represent the factors changing most during a diurnal cycle. Driven by the hypothesis that plants might experience further fluctuations during a diurnal cycle, potentially arising from complex interactions of the already modeled responses, we extended our models by incorporating additional starvation/perturbation experiments as explanatory variables. Using these additional experiments we sought to improve the prediction of the overall gene expression at a particular time point and used the gain in predictive power as an indicator whether a given experiment should be incorporated into the model.

Our models thus compare the behavior of wild-type *A. thaliana* to that of the *pgm* starchless mutant, and to wild-types in extended night conditions. We show that the *pgm* mutant suffers from nutrient depletion much earlier than wild-type plants, and that this can be predicted by gene expression patterns. Moreover, we show that, later in extended night conditions, wild-type plants respond in a way that has similarities to several different nutrient deprivation and stress conditions.

P436 Towards dissecting the Trans Golgi Network using proteomics and chemical genomics

Georgia Drakakaki, Mien van de Ven, Songqin Pan, Glenn Hicks, Natasha Raikhel

University of California, Riverside, California, USA

Membrane trafficking and associated signal transduction pathways are critical for plant development and responses to environment. These transduction pathways, including those for brassinosteroids and auxins, require endocytosis to endosomes and recycling back to the plasma membrane. A major challenge towards understanding these processes and their biological roles has been the highly dynamic nature of endomembrane trafficking.

To study components of the endomembrane system that are present in the Trans Golgi Network (TGN), we used a proteomic approach. Although proteomics has previously been used to characterize organelles such as mitochondria, chloroplast and nuclei, proteomics of the endomembrane system has not been a trivial task. We undertook an immunoisolation approach to separate TGN vesicles and analyze their cargoes. We have thus far successfully isolated SYP61 vesicles and identified several of their proteins.

In a complementary approach we employed chemical genomics to effectively study endocytosis and recycling, a process which occurs in a time frame of minutes. In a previous effort we isolated Endosidin-1 (ES1), a chemical that defines a SYP61- VHA-a1 compartment and revealed that PIN2, AUX1, and BRI1 use interactive pathways involving an early SYP61/VHA-a1 endosomal compartment (Robert *et al*, 2008, Proc Natl Acad Sci, USA 105: 8464). Continuing this effort, several pharmacological inhibitors that alter SYP61 localization will be presented.

P437 Shedding light upon the plant circadian clock: Models including explicitly the dynamics of the photoreceptor phyB

Christian Fleck1,2, Andrew Millar3, Eberhard Schafer2, Stefan Kircher2, Ferenc Nagy3

1Center of Biological Systems Analysis, 2University of Freiburg, Germany, 3University of Edinburgh, UK

Phytochromes mediate the physiological responses of plants that are induced by red light. In particular, key clock genes are regulated by phytochromes, providing a mechanism for them to reset the clock. However, the light input into the clock is treated in a simplified manner in current clock models without modelling a specific photoreceptor. We report on first results from an ongoing effort to extend the models of the plant circadian clock by explicitly accounting for the dynamics of the photoreceptor phyB.

P438 Light induced degradation of the transcription factor PIF3

Christian Fleck1,2, Sebastian Sonntag1,2, Stefan Kircher2, Eberhard Schafer2

1Center of Biological Systems Analysis, 2University of Freiburg, Germany

After the light induced conformational change phytochrome B is imported into the nucleus and binds to PIF3. This interaction leads to the degradation of PIF3. We report on experimental results showing the influence of phytochrome B on PIF3 and discuss mathematical models for the dynamics of the involved processes.

P439 The Arabidopsis circadian clock: Insights from mathematical modelling

Ozgur Akman1, John O'Neill1, David Rand2, James Locke3, Andrew Millar1

1Centre for Systems Biology, University of Edinburgh, UK, 2Systems Biology Centre, University of Warwick, UK, 3Applied Physics Division, California Institute of Technology, USA

The regulatory gene network underlying circadian oscillations in *Arabidopsis thaliana* has become a paradigm experimental system for quantifying the relationship between the structures of biochemical systems and their functional properties. By constructing mathematical models of the clock and analysing the models using techniques derived from nonlinear dynamics and mathematical control theory, we have established a theoretical framework for quantifying the clock's flexibility and robustness properties. This has yielded novel insights into the reasons why the Arabidopsis network has a considerably more complex architecture than the minimal negative feedback loop required for oscillatory behaviour.

Moreover, we have shown that the analytical tools developed have potential applicability to a broad range of cellular circuits, including clock networks in other organisms and interferon signalling pathways.

P440 Dissection of endomembrane sorting using bioactive chemicals

Stephanie Robert1, Georgia Drakakaki2, Michelle Brown2, Anna-Maria Szatmari1, Friml Jiri1, Russinova Eugenia1, Natasha V Raikhel2, Glenn R Hicks2

1VIB Department of Plant Systems Biology, Ghent University Technologiepark, Ghent, Belgium, 2Center for Plant Cell Biology and Department of Botany and Plant Sciences, University of California, Riverside, CA

Proteins are delivered to and recycled from the plasma membrane (PM) via endosomes, yet the nature of the dynamic compartments and pathways responsible for cargo and vesicle sorting and cellular signaling is poorly understood. To dissect specific recycling pathways, rapid-acting chemical affecters of proteins involved in vesicle trafficking, especially through endosomes, would be invaluable. Thus, we identified chemicals affecting essential steps in PM/endosome trafficking by utilizing the intensely localized PM transport at the tips of germinating tobacco pollen tubes. We screened diverse chemical libraries for chemicals that interfered with pollen germination and tip growth. We found that many had effects in Arabidopsis roots for which there are several well-characterized marker proteins that cycle to and from the plasma membrane. The compound endosidin 1 (ES1) interfered selectively with endocytosis of PIN2, AUX1 and BRI1

allowing us to define compartments in Arabidopsis root cells through which these markers transit during endocytosis.

These results indicate the value of using a chemical biology approach to understand mechanisms of endomembrane trafficking. We have now greatly expanded our chemical screening approach in order to obtain a suite of probes to dissect endomembrane trafficking and sorting processes.

Recent results from our screens will be discussed.

P441 Under the skin: Transcriptional subnetworks in the Arabidopsis root

Jalean J Petricka, Philip N Benfey
Duke University, Durham, NC, USA

In the era of 'omics approaches we are gaining knowledge of the overall structure of networks regulating plant development and response to the environment. Recent data arising from a combination of fluorescence activated cell sorting (FACS) of cell-type-specific fluorescent reporter lines with microarray experiments have led to resolution of these networks at the cellular level. Since proper tissue function relies on the ability of cells to acquire identity and differentiate appropriately, these data pertaining to cellular networks can be used to construct tissue networks. In this work we aim to discover how cells integrate transcriptional information into complex tissue processes in the ground tissue, which lies under the skin of the plant (the epidermis).

To identify transcriptional subnetworks in the ground tissue of the *Arabidopsis thaliana* root, we first mined expression data from root cell populations generated by Brady *et al* for transcription factors (TFs), known TF consensus binding sites, and clusters of genes that are significantly enriched in the root endodermis and cortex cell populations that together comprise ground tissue. Using these data and clusters we inferred a number of transcriptional subnetworks functioning in ground tissue. We have used chromatin immunoprecipitation followed by quantitative PCR (ChIP-qPCR) to biologically verify connections within these putative transcriptional subnetworks. We are now extending these studies to the whole genome level using ChIP followed by microarray studies (ChIP-chip) to put these networks into the larger context of the system. We plan to further extend these results and the subnetworks by determining the targets of TFs that are themselves targets in ChIP-chip experiments.

We are concurrently evaluating insertional mutant lines corresponding to the genes within these transcriptional subnetworks for defects in root development and response, specifically in the ground tissue.

Using these approaches we are unveiling the transcriptional circuitry of ground tissue that lies under the skin of the Arabidopsis root.

P442 An Arabidopsis genetical genomics approach to improve phytonutrient quality in Brassica vegetable crops

Wessel van Leeuwen1, Ric C H de Vos2, H K Kim3, Rob Verpoorte3, Ronny V L Joosen2, Kim Boutilier2, Frank Johannes4, Ritsert C Jansen4, Mark G M Aarts1
1Laboratory of Genetics, Wageningen University, Wageningen, The Netherlands, 2Plant Research International, Wageningen, The Netherlands, 3Division of Pharmacognosy, Leiden University, Leiden, The Netherlands, 4Groningen Bioinformatics Centre, University of Groningen

Brassica vegetables contain a wide variety of secondary metabolites that contribute in both positive and negative ways to their nutritional qualities. We use Arabidopsis as a model species to study the phytonutrient biosynthesis pathways of Brassicaceae to identify unknown regulatory or biosynthesis genes.

The metabolic differences of several accessions were determined by both LC-QTOF MS and ^{1}H-NMR, based on which we chose the genetically characterised segregating recombinant inbred line population Landsberg erecta x Kashmir-2 for our study. This population was grown hydroponically for four weeks. Pooled rosettes of six plants were used for metabolic profiling using both targeted and untargeted approaches. This metabolite survey focussed on the identification and quantification of phytonutrients such as glucosinolates, phenylpropanoids and flavonoids, folate, carotenoids and tocopherols. In addition to the metabolome analysis, the same leaf samples were used for gene expression analysis using a distant-pair microarray design. Using both the metabolite and transcriptome data as trait data we subsequently performed a QTL analysis.

The results of this analysis, focussing on glucosinolate biosynthesis will be discussed. All metabolome, transcriptome and QTL information will be combined to predict metabolic networks and find new regulators or biosynthesis genes. These genes, identified in Arabidopsis, will then be used to identify the corresponding *Brassica rapa* orthologues and develop molecular markers for breeding purposes.

P443 Large-scale analysis of Arabidopsis transcription reveals a basal co-regulation network

Osnat Atias1, Benny Chor2, Daniel A Chamovitz1
1Department of Plant Sciences, Tel Aviv University, Israel, 2School of Computer Science, Tel Aviv University, Israel

Analyses of gene expression data from microarray experiments has become a central tool for identifying co-regulated, functional gene modules. A crucial aspect of such analysis is the integration of data from different experiments and different laboratories. How to weigh the contribution of different experiments is an important point influencing the final outcomes. We have developed a novel method for this integration, and applied it to genome-wide data from multiple Arabidopsis microarray experiments performed under a variety of experimental

conditions. The goal of this study is to identify functional globally co-regulated gene modules in the Arabidopsis genome. Following the analysis of 21,000 Arabidopsis genes in 43 datasets and about 2 x 108 gene pairs, we identified a globally co-regulated gene network comprising about 10% of the Arabidopsis transcriptome. We identified clusters of globally co-regulated Arabidopsis genes that are enriched for known Gene Ontology annotations. Two types of modules were identified in the regulatory network: stable and unstable modules, which we further show to pertain to general and specialized modules, respectively. These modules were validated by comparison with the Genevestigator compendium of microarray experiments. Analyses of smaller subsets of data lead to the identification of condition-specific modules. The global co-regulation network facilitates the identification of novel gene modules and assignment of new functions to under-annotated genes. Our method for identification of gene clusters allows the integration of diverse microarray experiments from many sources and is general enough to apply to any set of microarray experiments, using any scoring function.

P444 Multiple alternative splicing events in individual transcripts; analyses using full-length cDNAs and tiling arrays

Kei Iida1, Shuji Kawaguchi1, Kousuke Hanada1, Kaoru Fukami-Kobayashi2, Atsushi Toyoda3, Yoshiyuki Sakaki4, Masatomo Kobayashi2, Motoaki Seki1, Kazuo Shinozaki1, Tetsuro Toyoda1

1RIKEN, Yokohama, Japan, 2RIKEN, Tsukuba, Japan, 3National Institute of Genetics, Mishima, Japan, 4Toyohashi University of Technology, Toyohashi, Japan

Alternative splicing (AS) is a mechanism by which multiple types of mature mRNAs are generated from a single pre-mature mRNA. In this study, we completely sequenced 1,800 full-length cDNAs from *Arabidopsis thaliana*, which had 5' and/or 3' sequences that were previously found to have AS events or alternative transcription start sites. Unexpectedly, these sequences gave us further evidence of AS events in their novel sequenced regions. In comparison with annotation models in TAIR, 601/1,800 transcripts showed novel AS events.

Current analysis showed that more numbers of transcripts have multiple (>= 2) AS events than known. We focused on the combination patterns of multiple AS events within individual transcripts. Interestingly, some specific AS event combination patterns tended to appear more frequently than expected. The two most common patterns were, A) alternative donor - 0~12 times of exon skips - alternative acceptor, and B) several times (~8) of retained introns. We also found that multiple AS events in a transcript tend to have the same effects concerning the length of the mature mRNA. Our current results suggest that certain *trans*-acting factors are responsible for generating AS profiles.

We are integrating tiling array results and other transcriptiome data with the knowledge of the AS events. We will show recent progress of study of AS profiles in *Arabidopsis thaliana*.

Iida *et al*, (2009) Analysis of multiple occurrences of alternative splicing events in *Arabidopsis thaliana* using novel sequenced full-length cDNAs. DNA res. (in press)

P445 ARTADE2.0: A mathematical integration of tiling array, CAGE and sequence data to elucidate the transcriptional systems dynamics of *Arabidopsis thaliana*

Shuji Kawaguchi1, Kousuke Hanada1,2, Kei Iida1, Yoshiki Mochizuki1, Akihiro Matsui2, Masanori Okamoto2, Motoaki Seki2, Kazuo Shinozaki2, Tetsuro Toyoda1

1Bioinformatics And Systems Engineering (BASE) division RIKEN, Yokohama, Kanagawa, Japan, 2Plant Science Center (PSC) RIKEN, Yokohama, Kanagawa, Japan

Several novel technologies allow an analysis of a transcriptome with a single experiment. For examples, tiling arrays are useful for an estimation of exon-intron structures. CAGE (Cap Analysis of Gene Expression) can measure expression levels of genes by taking the count of 5'end caps of mRNAs. These experiments must show genes, which are not known until today. However, gene findings by the use of only one technique are still incomplete since noisy signals are mixed in tiling arrays and the CAGE often picks up irregular caps and passes up appropriate caps. To generate meaningful results will require a synthetic approach to using these databases along with an embedded mathematical approach. Then, we propose a new statistical gene prediction method for Arabidopsis based on multi conditional tiling arrays and CAGE tags. The method is an improvement model of ARTADE (ARabidopsis Tiling-Array-based Detection of Exons, Toyoda and Shinozaki[1]) and estimates gene structures by considering Markov transitions of nucleotides and the correlation matrix of tiling arrays in multi conditions. Moreover, the CAGE is introduced into the method as valuable information for the prediction of 5' end. We used tiling arrays of eighteen conditions (55 experiments in total) and CAGE tags of four conditions for the gene prediction. The proposed method worked well for the prediction of 5'end and 3'end at more than 90% accuracy of TAIR8 genes. We also succeeded in funding more than a thousand unknown gene structures with the high quality of prediction.

Toyoda T and Shinozaki K. Tiling-array-driven elucidation of transcriptional structures based on maximum-likelihood and Markov models. The Plant Journal, 43(3), 611-621 (2005)

P446 A one-loop model of the *Ostreococcus tauri* circadian clock

Carl Troein1, Andrew Millar1,2

1Inst. of Mol. Plant Sci., University of Edinburgh, Edinburgh, UK, 2CSBE, University of Edinburgh, Edinburgh, UK

The circadian clock is a system of great importance to any organism whose life depends on light-dark cycles. In higher plants such as Arabidopsis, this oscillator is known to regulate the expression of a large number of genes, and modelling and experiments have revealed much

about how it works. The major components of the clock are genes with several homologues, and together they form a highly non-trivial system of interconnected feedback loops, making accurate modelling a great challenge. In contrast, in *Ostreococcus tauri*, a tiny alga with an exceptionally small genome, there are only a handful of homologues to known Arabidopsis clock genes, most importantly a single homologue of each of the genes *TOC1* and *CCA1*. The organism is nonetheless capable of temperature-compensated entrainment to light/dark cycles in both long and short days, as would be expected from a proper circadian clock.

We have modelled the Ostreococcus clock as a single negative feedback loop between *TOC1* and *CCA1*, and shown that this model fits with data from luciferase promoter and protein fusion experiments, both under light/dark cycles of various photoperiod and in constant light.

P447 Comprehensive analysis of Arabidopsis expression level polymorphisms with simple inheritance

Stéphanie Plantegenet1, Johann Weber1, Darlene R Goldstein2, Georg Zeller3, Cindy Nussbaumer1, Jérôme Thomas1, Detlef Weigel4, Keith Harshman1, Christian Hardtke1

1University of Lausanne, Lausanne, Switzerland, 2EPFL, Lausanne, Switzerland, 3Friedrich Miescher Laboratory of the Max Planck Society, Tübingen, Germany, 4Max Planck Institute for Developmental Biology, Department of Molecular Biology, Tübingen, Germany

In *Arabidopsis thaliana*, gene expression level polymorphisms (ELPs) between natural accessions that exhibit simple, single locus inheritance are promising quantitative trait locus (QTL) candidates to explain phenotypic variability. It is assumed that such ELPs overwhelmingly represent regulatory element polymorphisms. However, comprehensive genome-wide analyses linking expression level, regulatory sequence and gene structure variation are missing, preventing definite verification of this assumption. Here we analyzed ELPs observed between the Eil-0 and Lc-0 accessions. Compared to non-variable controls, 5' regulatory sequence variation in the corresponding genes is indeed increased. However, ~42% of all ELP genes also carry major transcription unit deletions in one parent as revealed by genome tiling arrays, representing more than 4-fold enrichment over controls. Within the subset of ELPs with simple inheritance, this proportion is even higher and deletions are generally more severe. Similar results were obtained from analyses of the Bay-0 and Sha accessions, using alternative technical approaches. Collectively, our results suggest that deleterious structural changes are a major cause for ELPs with simple inheritance, corroborating experimentally observed indel preponderance in cloned Arabidopsis QTL.

P448 Control of the accumulation of major flavonol glycosides by R2R3-MYB PRODUCTION OF FLAVONOL GLYCOSIDES regulators in adult *Arabidopsis thaliana* plants

Ralf Stracke, Matthias Keck, Antje Bluemke, Karsten Niehaus, Bernd Weisshaar

CeBiTec, Bielefeld University, Bielefeld, Germany

Flavonoid biosynthesis is the plant pathway for which probably the most is known with regards to its control, providing examples of what might be expected for the regulation of other metabolic pathways. In *Arabidopsis thaliana* the flavonol branch is under transcriptional control of the R2R3-MYBs PRODUCTION OF FLAVONOL GLYCOSIDE1 (PFG1/MYB12, PFG2/MYB11 and PFG3/MYB111). A combination of genetic and metabolite analysis approach was used to identify transcription factor gene-metabolite correlations of the flavonol metabolic pathway in different organs of adult *A. thaliana* plants. Using high performance thin layer chromatography (HPTLC) and flavonol specific diphenylboric acid 2 aminoethylester (DPBA) staining, flavonol glycoside end-product accumulation patterns have been analysed in wild-type and multiple R2R3-MYB PFG mutants in an organ- and development-dependent manner. This clearly demonstrates a differential influence of PFG1/MYB12, PFG2/MYB11 and PFG3/MYB111 MYB11/PFG2 on the spacial accumulation of specific flavonol derivatives.

P449 Using synthetic biology and a marine algae to simplify the study of light entrainment to the Arabidopsis circadian clock

Laura Dixon1, Florence Corellou2, François-Yves Bouget2, Andrew Millar1

1I.M.P.S. and C.S.B.E., University of Edinburgh, UK, 2Université Paris VI, Banyuls, France

Light is one of the most important environmental factors, not only for stimulating and maintaining plant growth and development but also for the co-ordination of these, and many other fundamental processes. This temporal co-ordination is mainly orchestrated through the circadian clock, an endogenous rhythm that can be entrained by light/dark cycles. The information within these light/dark cycles is not only duration but also the quality and quantity of light. Arabidopsis is known to require 8 of its photoreceptors to entrain the circadian clock, making the dissection of the network extremely complicated. Here we present two methods for the simplification of this complex network; firstly a synthetic biology approach and secondly using a less complex photosynthetic model organism *Ostreococcus tauri.*

Using a synthetic biology approach we have characterised a number of blue-light photoreceptors in the yeast *S.cerevisiae* that can be used to entrain or synchronise a genetic network in a predictable and controllable manner.

Secondly, the use of *O.tauri*, a pico-eukaryotic algae, enables not only the dissection of entrainment pathways in a clearer way, as it contains only two likely

photoreceptors, but also enables cell culture based manipulations. These approaches allow the identification and manipulation of entrainment mechanisms beyond those of the known photoreceptors: information, which can then be transferred to the study of Arabidopsis.

P450 Quantification of *BAK1* and *BRI1* receptors

G W van Esse1, J W Borst1, C Albrecht1, M de Gee2, J Molenaar2, S C de Vries1

1Laboratory of Biochemistry, Wageningen University, Wageningen, The Netherlands, 2Mathematical and Statistical Methods Group, Wageningen University, Wageningen, The Netherlands

The *Somatic Embryogenesis Receptor Kinase (SERK)* family consists of five homologous proteins involved in a variety of signalling pathways.[1] One of the members of this family, the BRASSINOSTEROID INSENSITIVE1 (BRI1)-ASSOCIATED KINASE1 (BAK1) receptor (also referred to as *SERK3*) is involved as a co-receptor in different signalling pathways. BAK1 interacts with Brassinosteroid Insensitive 1 (BRI1) in brassinosteroid signalling[2] and with Flagellin Sensitive 2 (FLS2) and the Elongation Factor Tu-receptor (EFR) in defense.[3] In addition, BAK1 is also involved in the mediation of cell death.[4] To study how BAK1 is functioning and divided over these pathways several approaches will be used. First, the amount of receptors will be quantified in planta. Second, the role of co-receptors and in particular BAK1 will be studied in each pathway separately. Finally, a mathematical model for the distribution of BAK1 over the pathways that employ BAK1 will be established. To this end, the amount of BAK1 and BRI1 receptors has been determined using quantitative fluorescence confocal microscopy. Further, the effect of co-receptor(s) on BRI1 signalling is studied in various mutant backgrounds using root length assays as an indicator for functional BRI1 signalling.

1 Albrecht *et al*, Plant Physiology, 2008, 148, 611
2 Nam and Li, Cell, 2002, 110, 203
3 Chinchilla *et al*, 2007 Nature, 448, 497
4 Kemmerling *et al*, Current Biology, 2007, 17, 1116

P451 Transcription regulatory programs of cell cycle control in the root vascular tissues

Sarit Weissmann1, Je-Gun Joung1, Zhangjun Fei1,2, Ykä Helariutta3, Ji-Young Lee1,4

1Boyce Thompson Institute for Plant Research, Ithaca, NY, USA, 2USDA Robert W. Holley Center for Agriculture nd Health, Ithaca, NY, USA, 3Institute of Biotechnology, University of Helsinki, Helsinki, Finland, 4Department of Plant Biology, Cornell University, Ithaca, NY, USA

Plant postembryonic development and growth occur from localized regions, the meristems. The root meristem consists of several populations of dividing precursor cells. Specification and proliferation of these precursor cells are determined by dynamic gene regulatory networks. These regulatory networks exhibit a specific combination of transcription factors (input) that determines the gene expression profile (output) of each cell type or tissue, at a specific developmental stage. How the regulatory networks control the transition from undifferentiated precursor cells to differentiated cell types is the main question of our investigation. We used high-throughput gene expression profiling in the Arabidopsis root and probabilistic models to infer gene regulatory modules that would identify the key transcription factors involved in determining the vascular cell type identity and proliferation. Our analysis revealed several putative regulatory modules specific to various precursor cell types in the meristem of the Arabidopsis root. Interestingly, several modules over-represented in the vascular cell type precursors were found to be involved in cell cycle regulation. Knock-out lines of potential transcriptional regulators of these modules exhibited irregular cell divisions of the vascular cell type precursors in the root. Our results demonstrate the applicability of high-throughput module construction in multicellular organisms and the potential significance of cell cycle regulation in vascular tissue development.

P452 Composite module analysis using BIOBASE ExPlain™ plant identifies factors which may influence *ms1* pollen and tapetum developmental phenotypes

Raymond DiDonato1, Ruy Jauregui2, Alexander Kel2, Edgar Wingender2

1BIOBASE corporation, USA, 2BIOBASE GmbH, Germany

Male sterility1 (MS1) is a PHD-finger transcription factor that regulates pollen and tapetum development in Arabidopsis. Previous microarray analysis studies identified genes that were misregulated in the *ms1* mutant (Alves-Ferreira *et al* 2007. Global expression profiling applied to the analysis of Arabidopsis stamen development. Plant Physiol. 145(3):747-62). Functional analysis identified lipid metabolism, transcription factor activity, secondary metabolism, and the external encapsulating structure as overrepresented functional classes within this set of misregulated genes. Moreover, genes involved in abscisic acid (ABA) and ethylene signaling were differentially expressed, suggesting a role for these hormone signaling pathways in observed *ms1* phenotypes. Using the BIOBASE Knowledge Library Plant Edition (BKL-Plant) and ExPlain-Plant Analysis System, we re-analyzed genes differentially expressed in the whole inflorescence of the *ms1* mutant to identify possible transcriptional regulators and upstream signaling events influencing gene expression and the *ms1* phenotype. We also re-examined functional analysis by GO classification and mapped the gene set to pathways, in order to more fully understand downstream events affected in the *ms1* mutant. MATCH analysis combined with composite module analysis (CMA) revealed that RAV1 and ARR10 binding sites, as well as sites similar to those bound by tomato ASR1, were overrepresented in the promoters of differentially expressed genes. Upstream pathway analysis suggested cross-talk with the ethylene signaling pathway through the phosphorelay protein AHP1, which interacts with ARR10 and may signal through ETR1, confirming the influence of ABA and ethylene signaling on differential gene expression in *ms1* and identified upstream factors mediating

this effect, and suggesting a role for cytokinin signaling pathways as well. In addition to previously identified over-represented functional classes, various cell wall modification and carbohydrate metabolic processes and activities, as well as endomembrane system, were identified as likely to be affected in the *ms1* mutant.

P453 Chloroplast proteome analysis: New insights into intracellular trafficking

Sylvain Bischof1, Thomas Wildhaber1, Trösch Raphael1, Felix Kessler2, Wilhelm Gruissem1, Sacha Baginsky1

1Institute of Plant Sciences, ETH Zurich, Switzerland, 2Laboratoire de Physiologie Vegetale, Universtity of Neuchatel, Switerland

The biogenesis and functionality of plastids requires the import of several thousand nuclear-encoded proteins. The proper targeting and the selective import of cytosolic pre-proteins rely on a plastid protein import machinery consisting of TOC (translocon at the outer chloroplastic membrane) and TIC (translocon at the inner chloroplastic membrane) protein complexes (1). Recent data suggested furthermore, that in addition to TOC/TIC-mediated import, alternative routes exist that direct plastid proteins through the secretory pathway (2). High-throughput proteomics data support this view and suggest, that intracellular protein trafficking may be more complex than previously anticipated (3-4). We therefore analyzed the proteome of two plastid protein import mutants, *ppi1* and *ppi2*, lacking components of the plastid protein import machinery in order to assess the contribution of alternative import routes to plastid proteome composition. More than 1500 different proteins were identified and quantified from isolated plastids. Groups of co-regulated genes were assembled by K-means clustering and the transit peptide composition of the members in each cluster analyzed. Overall, the protein accumulation in the different mutants was surprisingly similar suggesting basic robustness principles and limited plasticity for the assembly of organellar proteomes. In order to further characterize chloroplast protein import in the different mutants, we systematically searched for N-terminal acetylated peptides in genome-scale WT, *ppi1* and *ppi2* proteomics data. These analyses revealed the accumulation of precursor proteins in the TOC159 deficient mutants (*ppi2*), probably as a result of the impaired import reaction. The plastid precursor proteins enter into the two-step cytosolic methionine removal/acetylation pathway. Interestingly, the import of many other plastid proteins was not affected by the mutation and the proteins accumulated in their mature, processed form. We discuss our observations in the context of protein import specificity.

P454 Global control of rhythmic gene expression by the transcription factor LHY

Sally Adams, Siren Veflingstad, David Rand, Bärbel Finkenstädt, Sashca Ott, Isabelle Carré

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

Circadian clocks have evolved in a diverse range of organisms enabling them to regulate biochemical and physiological processes in anticipation of daily environmental changes. At the core of the clock is the central oscillator, which consists of multiple inter-locked transcriptional feedback loops responsible for generating circadian rhythms. Input pathways allow the clock to be set in response to environmental signals, whilst output pathways link the oscillator mechanism to the plethora of biological processes under circadian control.

The LHY transcription factor acts as part of the central oscillator in higher plants and also regulates the expression of a multitude of downstream output genes. Although all known LHY targets exhibit oscillatory patterns of transcript accumulation, the phase of expression varies from gene to gene. The aim of this project is to elucidate how a simple rhythmic pattern of LHY is able to regulate such varied temporal patterns of expression. LHY binds specific short sequences (EE and CBS), which are present in many target gene promoters. However, these elements are not sufficient to explain the timing of gene expression suggesting additional contextual information is important, such as different affinities of LHY to different promoters and/or interactions between LHY and other regulatory proteins.

To test these hypotheses we will use a multi-disciplinary approach. Using ChIP-seq technology and bioinformatics we will identify all LHY binding sites in the genome. With this invaluable source we can then test the affinity of LHY for different classes of binding sites and identify co-factors using a comparative genome approach. Furthermore, we will generate time-course data to study changes in LHY protein levels and LHY binding to key promoters, as well as transcriptional activation and transcript levels of key target genes across the circadian cycle. All the experimentally observed data will then be used to generate mathematical models of gene regulation. In conclusion, not only will this work shed further light on the complexities of the plant circadian clock, it will also provide an excellent system to answer the fundamental question, how can a cell regulate varied and robust patterns of gene expression with a limited set of transcription factors?

Hot Topics

P455 Genetic mechanisms of hybrid incompatibility in *Arabidopsis thaliana*

Roosa Laitinen, Kirsten Bomblies, Helena Boldt, Marco Todesco, Detlef Weigel

Max Planck Institute for Developmental Biology, Tuebingen, Germany

A survey of the F1 offspring of 1487 crosses in Arabidopsis revealed 25 F1 hybrid necrosis cases. These include at least 5 genetically independent systems that we are currently analysing. The first gene underlying hybrid incompatibility that has been cloned turned out to be a homolog of NB-LRR R-genes, a gene class involved in disease resistance in plants (Bomblies *et al* 2007). Together with expression profiling and presence of cell death it was concluded that several of the hybrids suffer

from autoimmunity. Interestingly, despite most of the cases involving 2-3 loci, as the basic Dobzhansky-Muller model assumes, there are some cases in which the genetic incompatibility seems to be due to heterozygous disadvantage at a single locus. I will present one such case, which appears to be responsible for incompatibility in several crosses. Other alleles at this locus cause necrotic lesions in inbred strains, providing a connection between incompatibility between strains and potential fitness tradeoffs within strains. To further explore the genetic landscape leading to hybrid weakness, we have screened F2 populations for recessive incompatibilities. I will present a situation where F2 plants are small and purple, while F1 plants have an altered inflorescence habit. The F1 and F2 phenotypes are linked and map to two epistatically acting loci. Hybrid weakness is found in many flowering plants and could lead to reproductive barriers within populations. Using *Arabidopsis thaliana* as a model system we are able to illuminate the very early steps of divergence when species are not yet separated.

Bomblies K, Lempe J, Epple P, Warthmann N, Lanz C, Dangl JL and Weigel D. Autoimmune response as a mechanism for a Dobzhansky-Muller-type incompatibility syndrome in plants. PLoS Biol. 2007: 5(9):e236.

P456 Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose 6-phosphate

Lucia F Primavesi1, Cátia Nunes2,3, Anabela Bernardes da Silva2, Pedro M Fevereiro3, P John Andralojc1, Alfred J Keys1, Astrid Wingler4, Mitul Patel5, Ram S Misra5, Ben G Davis5, Matthew J Paul1

1Rothamsted Research, Harpenden, UK, 2Universidade de Lisboa, Faculdade de Ciencias, Lisboa, Portugal, 3Instituto de Tecnologia Quemica e Biologica, Universidade Nova de Lisboa, Oeiras, Portugal, 4University College London, UK, 5University of Oxford Chemistry Research Lab, Oxford, UK

Trehalose 6-phosphate (T6P) is a proposed signalling molecule in plants, yet how it signals was not clear. Recently we showed that T6P is an inhibitor of SnRK1 of the SNF1-related group of protein kinases (Zhang *et al* 2009 Plant Physiology 10.1104/pp.108.133934). These conserved kinases perform a fundamental role in transcriptional, metabolic and developmental regulation in response to energy limitation and starvation of carbon source. Inhibition of SnRK1 by T6P is strongest in growing tissues but absent in mature leaves consistent with a model where T6P inhibits SnRK1 to activate processes associated with growth. In seedlings the inhibition of SnRK1 by T6P activates scores of genes associated with biosynthetic processes. We propose that T6P, being synthesised from core metabolites UDPG and G6P, signals their availability and through inhibiting SnRK1 activates downstream biosynthetic processes that use these core intermediates. Studies using immunoprecipitation and enzyme kinetics show that the inhibition is mediated by a factor, which is physically separable from the heterotrimeric SnRK1 complex. We are using protein purification to isolate and identify this factor.

P457 Design and analysis of ChIP-Seq experiments in plants: A systematic comparison of ChIP-Seq and ChIP-chip for APETALA2 (AP2), FD, and SCHLAFMÜTZE(SMZ)

Levi Yant, Felix Ott, Heike Keller, Detlef Weigel, Markus Schmid

Max Planck Institute for Developmental Biology, Tubingen, Germany

ChIP-Seq couples chromatin immunoprecipitation (ChIP) to ultra high throughput massively parallel sequencing to create *in vivo* genome-wide maps of protein-DNA interactions. Rapidly eclipsing ChIP-chip, which employs tiling arrays, ChIP-Seq promises higher positional resolution and decreased expense.

We generated ChIP-Seq and ChIP-chip datasets for three *Arabidopsis thaliana* transcription factors: *AP2, FD,* and *SMZ*. Coding regions were fused to GFP and ChIP was performed using identical antibodies to precisely isolate differences in DNA binding specificity. All lines recapitulated untagged phenotypes.

All experiments yielded high confidence datasets. For example, among the best bound FD targets by ChIP-chip were MADS-gene loci *SEPALLATA3 (SEP3)*, *APETALA1 (AP1)*, and *FRUITFULL (FUL)*. *AP2* and *SMZ* bound several other miR-172-targeted *AP2*-domain proteins, among which negative feedback regulation was observed, but until now it was unknown whether this was direct.

To determine how ChIP-Seq compared with ChIP-chip, we performed systematic comparisons. ChIP-Seq sequencing was performed on an Illumina 1G genome analyzer. Between 2 and 9 million high quality reads were mapped for each sample. With such numbers we obtained >90% coverage to the nonrepetitive genome. Several ChIP-Seq analysis methods were directly compared, including a novel program we designed specifically for *Arabidopsis thaliana* ChIP-Seq data. ChIP-Seq and ChIP-chip datasets were largely consistent, especially for high confidence(FDR Q<10^-20) binders: for example, FD binding to MADS-gene loci *SEP3, AP1*, and *FUL* was detected by both methods with high confidence.

Systematic comparison of the relative utility of technical and biological replicates and requirements of genome sequencing depth will be presented in detail.

P458 Trehalose metabolism and sugar signalling in plants

John Lunn

Max Planck Institute of Molecular Plant Physiology, Golm Germany

Trehalose metabolism was once thought to be uncommon in higher plants, until the discovery of trehalose-phosphate synthase (TPS) and trehalose-phosphatase (*TPP*) genes in *Arabidopsis thaliana* led to a complete reappraisal of its importance. Genome sequencing and

mutant analyses have now shown that trehalose metabolism is not only widespread within the plant kingdom, but also that it is essential for normal plant growth and development. Plants with altered trehalose metabolism show marked morphological and physiological phenotypes, which are linked to changes in the level of trehalose 6-phosphate (Tre6P), the intermediate of trehalose synthesis, rather than to trehalose itself. Using an LC-MS/MS-based assay, we found that the amount of Tre6P in plant tissues reflects changes in the level of sugars, particularly sucrose, leading us to propose that Tre6P acts as a signal of sucrose status.[1] The upstream signalling pathway between sucrose and Tre6P is being investigated. Inhibitor studies showed that protein synthesis is required for the sucrose-induced rise in Tre6P, and that protein turnover could also be involved in the response. We are also testing the hypothesis that Tre6P acts as a signal of sucrose availability in meristematic regions and developing organs, where it could be integrated with other signalling pathways, e.g. auxins and cytokinins, to regulate the growth and development of the plant.

Lunn *et al* (2006) Biochemical Journal 397; 139-148.

P459 Phenotypic buffering in Arabidopsis: A genetical genomics approach

Joost J B Keurentjes1, Jingyuan Fu2

1Laboratory of Genetics and Plant Physiology, Wageningen University, The Netherlands, 2Groningen Bioinformatics Centre, University of Groningen, The Netherlands

Phenotypic diversity within species can be the consequence of heritable genomic variation. The resulting complex trait variation can be effectively analyzed in mapping populations identifying quantitative trait loci (QTL) causal for the observed variation. However, many intermediary steps separate the DNA sequence polymorphisms from the eventual phenotype and genetic regulation can act on each of these subsequent steps. Modern technologies (transcriptomics, proteomics, and metabolomics) now enable us to follow the path from genotype to phenotype in great detail on every level. Similar to classical quantitative traits, variation in gene expression, protein and metabolite abundance can be subjected to QTL analysis, an approach known as genetical genomics. We have performed the first system-wide genetical genomics study of molecular variation in a model organism, in which we integrate transcript, protein and metabolite data with publicly available phenotypic data from a population of recombinant inbred lines (RILs) of *Arabidopsis thaliana* (Fu *et al*, 2009). Although the parental lines of the population differed in at least 500,000 SNPs expression QTLs (eQTLs) were detected for only 5000 genes, indicating that the majority of SNPs are neutral.

Moreover, only a handful of these genetic effects were propagated to the phenotype level. These results suggest that much of the genetic variation is buffered along the way from genotype to phenotype. The findings are in agreement with robustness theories which state that organisms are buffered against perturbations to retain optimal performance in stable environments. At the same time robustness allows the accumulation of mutations which might become beneficial in changing environments.

Fu, J., Keurentjes, J.J.B., Bouwmeester, H., America, T., Verstappen, F.W., Ward, J.L., Beale, M.H., de Vos, R.C., Dijkstra, M., Scheltema, R.A., Johannes, F., Koornneef, M., Vreugdenhil, D., Breitling, R., and Jansen, R.C. (2009). System-wide molecular evidence for phenotypic buffering in Arabidopsis. Nat Genet 41, 166-167.

P460 Novel pathways for recombination in plants: Roles for DNA ligases

Wanda M Waterworth1, Georgina E Drury1, Jaroslav Kozak2, Karel J Angelis2, Clifford M Bray3, Christopher E West1

1University of Leeds, Leeds, UK, 2Institute of Experimental Botany, Prague Czech Republic, 3University of Manchester, Manchester, UK

The DNA repair and recombination pathways operative in plant cells are important in the plant response to environmental stresses including increased UVB, drought and heavy metal pollution. For biotechnology, manipulation of these recombination pathways can also be used to both promote improved gene targeting frequencies in higher plants and meiotic recombination frequencies, important in plant breeding. Accumulating evidence indicates that in addition to the well established HR (Homologous Recombination) and NHEJ (Non Homologous End Joining) pathways, plants possess novel components and pathways for repair of cytotoxic double strand braks (DSBs). Components of these novel illegitimate recombination pathways are now under investigation in our lab using a combination of molecular genetic, biochemical and cell biology approaches.

The repair of both single and double DNA strand breaks requires the activities of DNA ligase enzymes, which are crucial for the maintenance of nuclear, mitochondrial and plastid genome stability. Eukaryotic organisms possess multiple DNA ligases with distinct roles and we have characterised the three DNA ligase genes expressed in Arabidopsis (*AtLIG1, AtLIG4*, and plant-specific *LIG6*). AtLIG1 has roles in both DNA repair and replication and is indispensable for cell viability. In mammals, the roles of LIG1 in excision repair pathways is well characterised, but roles in DNA double strand break repair are only recently becoming apparent. In our studies using Comet assay analysis we identified a role for AtLIG1 in an NHEJ–independent mechanism that repairs the bulk of DNA DSBs. This pathway displays very rapid repair kinetics, but this repair is significantly reduced in plants silenced for *AtLIG1* gene expression.

Ongoing analysis of Arabidopsis DNA ligases is focussed on the characterisation of their interacting protein partners and physiological roles and importance in novel plant DNA repair and recombination pathways.

P461 Mutations in Arabidopsis *AGO1* and *ZLL* antagonistically affect miRNA and siRNA pathways

Allison Mallory1, Annika Hinze2, Matthew Tucker2, Nicolas Bouché3, Virginie Gasciolli1, Taline Elmayan1, Dominique Lauressergues1, Hervé Vaucheret1, Thomas Laux4

1Laboratoire de Biologie Cellulaire, Institut Jean-Pierre Bourgin, INRA, Versailles, France, 2Faculty of Biology, University of Freiburg, Freiburg, Germany, 3Station de Genetique et Amelioration des Plantes, Institut Jean-Pierre Bourgin, INRA, Versailles, France, 4Faculty of Biology, Freiburg Institute of Advanced Studies, University of Freiburg, Freiburg, Germany

Arabidopsis ARGONAUTE (AGO) proteins AGO1 and ZWILLE/PINHEAD/AGO10 (ZLL) act in miRNA- and siRNA-mediated gene regulation. Analysis of miRNA-mediated target repression and siRNA-directed posttranscriptional gene silencing in *ago1 zll* double mutants revealed that loss of ZLL function re-establishes at least of subset of miRNA and siRNA pathways compromised in hypomorphic *ago1* mutants, suggesting that AGO1 and ZLL antagonistically affect small RNA-mediated repression. Swapping of the conserved protein domains of AGO1 and ZLL revealed that at least one conserved domain is interchangeable between both proteins whereas the other conserved domains provide specificity to AGO1 and ZLL protein function. The biological implications of AGO1 and ZLL antagonism and the ecotype-specific functions of ZLL during small RNA-directed posttranscriptional silencing also will be presented.

P462 Conserved post-translational mechanisms in plant and animal clocks identified by targeted chemical screening

G van Ooijen1, J S O'Neill1, F Correlou2, F Y Bouget2, A J Millar1

1Centre for Systems Biology, University of Edinburgh, Edinburgh, UK, 2Centre National de la Recherche Scientifique, Universite Paris VI, Banyuls sur Mer, France

Circadian rhythms in eukaryotes are generally considered to comprise of interlinked feedback loops that control a wide variety of vital processes. At the core of the Arabidopsis clock, CCA1 and LHY are morning-expressed transcription factors acting in a feedback loop on the evening-expressed regulator TOC1. Experimentation and mathematical modeling have refined this single-loop model, resulting in a network of multiple intertwined pathways. To better understand this complex clockwork, the smallest known free-living eukaryote *Ostreococcus tauri* has been developed as a novel circadian model organism, where the Arabidopsis clock components CCA1, LHY, and TOC1 are represented as single copy genes, and in which pharmacological approaches are greatly facilitated.

A key result is that significant portions of the daily cycle are insensitive to transcriptional inhibition. Although core clockworks are thought to rely heavily on transcriptional/translational feedback loops, post-translational mechanisms have increasingly been shown to regulate clock properties throughout the eukaryote domain. We therefore analysed the effects of inhibition of evolutionarily ancient signalling targets such as calcium, cAMP, phosphatases, and kinases that all have been reported to regulate period in other eukaryotic model organisms.

The different identity of core clock genes has fed the popular belief that clock mechanisms differ across kingdoms. Our data show that the contributions of post-translational mechanisms to biological clocks are conserved and might comprise the mechanistic core of circadian rhythms across the tree of life, from humans to the simplest plant model Ostreococcus. We anticipate that the Ostreococcus system may similarly accelerate research into other basic aspects of plant biology.

P463 Aberrant mRNAs in the plant nucleolus

Sang Hyon Kim1,2, Dominika Lewandowska1, Gillian Clark1, Craig Simpson1, Ali Pendle3, Olga Koroleva3,4, Peter Shaw, John Brown1,5

1Scottish Crop Research Institute, Dundee, UK, 2Myongji University, Kyeongki-do, Korea, 3John Innes Centre, Norwich, UK, 4University of Reading, Reading, UK, 5University of Dundee@SCRI, Dundee, UK

The nucleolus is involved in a range of RNA/RNP processing and assembly functions, cell cycle control and stress responses. We previously identified plant exon junction complex (EJC) proteins in a proteomic analysis of Arabidopsis nucleoli and confirmed the nucleolar association of these and other EJC proteins by localisation of GFP-protein fusions in Arabidopsis cells. cDNA libraries from mRNA of whole cells, purified nuclei and nucleoli contained fully spliced transcripts, transcripts from single exon genes and aberrant mRNAs (incompletely spliced, alternatively spliced or mis-spliced transcripts). The nucleolar library contained all three types of mRNAs but was highly enriched in aberrant mRNAs compared to nuclear or whole cell libraries. Analysis of transcript levels in multiple nucleolar and nucleoplasmic preparations by RT-PCR confirmed the presence of mRNAs and the enrichment of aberrant mRNAs in the plant nucleolus. The majority of the aberrant mRNAs in the Arabidopsis nucleolus contained premature termination codons (PTCs) and were potential substrates for nonsense-mediated decay (NMD) based on rules established in both mammalian and plant systems. To demonstrate that these transcripts are turned over by NMD, we analysed transcript levels in the *upf1-5* and *upf3-1* mutants. By RT-PCR we were able to demonstrate that, for at least some transcripts, the same RT-PCR products which were enriched in the nucleolus were also increased in the *upf* mutants and were therefore NMD-sensitive. GFP fusions of UPF1, UPF2 and UPF3 showed that UPF3 localised to the nucleolus, UPF2 to the nucleolus and cytoplasm (but not the nucleoplasm), and UPF1 to the cytoplasm.

Taken together, these results suggest that the plant nucleolus has a novel function in mRNA biogenesis and nonsense-mediated decay.

P464 High resolution nucleosome positioning in Arabidopsis

Sarah Usher, Smita Kurup, Paul Verrier, Stephen Powers, Graham J King

Rothamsted Research, Harpenden, UK

Nucleosomes are the basic structural component of chromatin. Consisting of ~147 bp DNA wrapped around a histone octomer, they are separated by linker DNA and a linker-associated histone. While the positioning of nucleosomes is not determined by a 'consensus DNA sequence', it is affected by low amplitude signals associated with various sequence preferences. Due to variation in linker length, the local distribution of nucleosomes on a genome has profound consequences for higher-level organisation of chromatin and functional behaviour.

Until recently there has been virtually no information on positioned nucleosomes in Arabidopsis, or indeed any plant. Since genome architecture and sequence composition differ between species, the rules governing nucleosome positioning and the local and global distribution are expected to differ across taxa. We have used methods of increasing resolution to identify positions of cross-linked mono- and di-nucleosomes across the entire Arabidopsis genome. This now allows us to gain insights into the sequence composition and physical characteristics of DNA within nucleosomes, and to test the accuracy of existing nucleosome prediction models (mostly dervived from yeast and animal genomes) when applied to Arabidopsis.

Establishing the genomic distribution of nucleosomes enables us to explore regional variation in linker length and the interaction with epigenetic marks, in the context of gene structure and transcriptional activity.

Paired-end Solexa sequencing has provided good coverage of the Arabidopsis genome and, together with data derived from 454 sequencing and high-resolution tiling microarrays, enables us to study specific aspects of nucleosome organisation in relation to chromosomal organisation, gene structure/function, and epigenetic marks. This provides a sound basis for a wide range of detailed comparative studies focused on specific stages of plant development, genotype x environment interactions and recombination.

P465 Ubiquitin ligase knockout makes young plants feel old – an ideal inducible model system to study onset and progression of senescence and cell death

Gabriele Drechsel, Katja Vogelmann, Johannes Bergler, Stefan Hoth

Molecular Plant Physiology, FAU Erlangen, Germany

Senescence is the final stage of plant development. Leaf senescence gives rise to impressive fall foliage in deciduous trees and is necessary for the death of leaves in annual plants. The underlying processes are developmentally and genetically well-defined. Tight control of senescence guarantees optimal recycling of resources from senescing leaves into young leaves or seeds to maximize growth and reproductive capacity. Molecular events during leaf senescence have previously been studied in aging, but also in dark-treated, stressed, and detached leaves.

We have recently identified Arabidopsis mutants defective in the E3 ubiquitin ligase gene *SAUL1* (Senescence-Associated Ubiquitin Ligase1, also named *PUB44*) that show induction of leaf senescence at any developmental stage when transferred to low light conditions. This inducible model system allows us to study the cellular and molecular events regulating onset and progression of senescence in a highly uniform plant population and independently of the developmental age.

To study the course of molecular events in regulation of leaf senescence, we have grown Arabidopsis wild-type and *saul1* mutant seedlings for two weeks under permissive light conditions, and have then induced senescence in *saul1* mutants by transferring the seedlings to low light. Samples were taken at narrow time intervals after transfer to investigate ABA levels that were elevated in *saul1* mutants and gene expression changes on a kinetic level. To this end, we have performed microarray analyses on samples taken very early after transfer to low light and thus identified genes that may encode key regulators for the onset of senescence and cell death. Transcript levels of these genes including senescence regulatory components such as *WRKY53* are up- or downregulated after a few hours only. In conclusion, *saul1* mutant plants are an ideal model system to study onset and progression of senescence and cell death on the level of organs, tissues and cells, but also on the level of organelle deterioration, solute transport and molecular signalling.

P466 *b1* paramutation: The heritable transfer of epigenetic information in *trans*

Maike Stam, Marieke Louwers, Max Haring, Rechien Bader

Swammerdam Institute of Life Sciences, University of Amsterdam, Amsterdam, The Netherlands

We investigate the molecular mechanism underlying gene regulation in *trans*. As a modelsystem we study paramutation, a mitotically and meiotically heritable change in gene expression induced by allele interactions in trans. We examine paramutation at *b1*, a regulatory gene of the maize pigmention pathway. The low expressed B' epiallele imposes its low transcription rate onto the high expressed B-I epiallele in trans. Recent data indicate a role for RNA in paramutation, but suggests that siRNAs are not sufficient. We hypothesize a role for physical interactions in addition.

Seven tandem repeats, ~100 kb upstream of the *b1* coding region, are essential for trans-inactivation and for high *b1* expression (Stam et al 2002 Genes & Dev). Enhancement of *b1* expression is tissue-specific and associated with H3ac and nucleosome depletion at the B-I hepta-repeat. B' is expressed at a low level and its repeats DNA and H3K27 methylated in a tissue-independent manner. The B' repeats do however also show tissue-specific nucleosome eviction and H3K9me2, indicating tissue-specific reinforcement of silencing. Our data indicate that DNA methylation is involved in the capacity of the B' allele to trans-inactivate B-I. H3ac and

H3K9me2 on the other hand are involved in tissue-specific regulation of *b1*.

3C technology identified tissue-specific and expression level-specific physical interactions at *b1* (Louwers *et al* 2009 Plant Cell). Upon tissue-specific activation of b1, the hepta-repeat and *b1* promoter physically interact at the high expressed B-I, but also at the low expressed B' locus, indicating a role in the tissue-specific regulation of *b1*. High *b1* expression is mediated by a multi-loop structure; besides the hepta-repeat, other sequence regions physically interact with the transcription start site as well, and these interactions are epiallele- and expression level-specific. Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE) uncovered multiple of these interacting regions as potentially regulatory. The examination of chromatin looping adds a new dimension to the study of gene regulation in plants.

P467 Inducible breaks: Generating a transgenic system to study DNA repair in somatic tissues

Georgina E Drury, Wanda M Waterworth, Christopher E West

University of Leeds, UK

Of the many kinds of DNA damage, the double strand break (DSB) is important to study as it is the most cytotoxic lesion. DSBs occur during DNA replication and through oxidative stress, and are essential for the exchange of genetic material on sister chromatids during meiosis. Repair of DSBs in eukaryotes is mediated by non-homologous end joining and homologous recombination. These recombination pathways are essential for cell viability in response to genotoxic stress and determine the way a transgene integrates into the genome.

In order to study DSB repair, breaks are experimentally induced by ionizing radiation and radiomimetic chemicals such as bleomycin. However such treatments are likely to induce other forms of DNA and cell stress, and the number of DSBs induced and their whereabouts in the genome can not be precisely determined. Work is underway to establish Arabidopsis lines in which DSBs are induced by meganucleases, at specific recognition sites. An approach that has already been successfully applied in yeast and human cells, it will allow us to characterize the molecular events that occur at a specified break in the genome. We are investigating the repair of a DSB induced by ISce-I or IPpoI in an inducible system, in which the meganuclease translocates from the cytoplasm to the nucleus following exposure to a hormone inducer. Once inside the nucleus, endonuclease activity will generate a DSB and induce or recruit DNA repair pathways specific only to this kind of damage. I-Sce I recognises an 18-base pair sequence that is not present within the Arabidopsis genome, thus the site has been introduced in a separate transformation event. In contrast, IPpoI has a 15bp recognition site that is present in the Arabidopsis genome, enabling the study of DSB repair of an endogenous plant gene.

The early events in DNA damage signaling in plants remain relatively uncharacterized. Development of an inducible breaks system will provide new insight into repair kinetics and the molecular events occurring during DSB detection and repair.

P468 A role for Argonaute5 in *Arabidopsis thaliana*

Laura Wallbridge, Paola Fedito, Ericka Havecker, Thomas Hardcastle, David Baulcombe

University of Cambridge, Department of Plant Sciences, Cambridge, UK

Arabidopsis thaliana contains ten Argonaute (AGO) proteins that have been grouped into three phylogenetic clades. The first clade contains AGO1, AGO10/Pinhead/Zwille and AGO5; the second group AGO7, AGO2 and AGO3 and the third clade, AGO4, AGO6, AGO8 and AGO9. AGO proteins selectively bind different subsets of a total population of 21-24nt small RNAs based on length, 5' nucleotide composition, and possibly other unknown factors. The resultant silencing complexes are involved in multiple and diverse silencing pathways involving microRNAs, trans-acting small interfering small RNAs and other types of small interfering RNAs. These gene-silencing pathways result in guided mRNA cleavage, translational arrest, transcriptional repression or RNA-directed DNA methylation resulting in DNA and chromatin modifications.

Previously published results as well as our own deep sequencing of AGO5 associated small RNAs indicate that AGO5 preferentially binds 24nt siRNAs with a 5' terminal cytosine. Based on its ability to bind 24nt small RNAs we proposed that AGO5 could influence small-RNA-directed DNA methylation (RdDM). Our data are consistent with this idea but unlike RdDM involving the AGO4 clade, the AGO5 effect is at CG motifs rather than C residues in a CNG or CNN context. SAGE sequencing of the *ago5* mutant transcriptome showed upregulation of coding and non-coding RNAs including those for which there was reduced methylation of their DNA in the mutant. The loss-of-methylation phenotype in the *ago5* mutant could be due to an AGO5 role in either transcriptional (like the AGO4 clade) post-transcriptional silencing (like the AGO1 clade) gene silencing, or both. Our alignment of the ten AGO proteins from *Arabidopsis thaliana* with orthologous AGO proteins from other plant species leads us to conclude that AGO5 diverges from the AGO1-clade of plant AGOs to form its own sub-clade.

We hypothesize that AGO5 has a unique role, non-redundant with either the Arabidopsis AGO1 or AGO4 clades, in RNAi directed DNA methylation.

P469 SUPPRESSOR OF ABI3-5 (SUA) regulates alternative splicing of the seed maturation gene *ABI3*

Matteo Sugliani, Vittoria Brambilla, Maarten Koornneef, Wim Soppe

Max Planck Institute for Plant Breeding, Koln, Germany

The ABSCISIC ACID INSENSITIVE 3 (ABI3) transcription factor has an essential role during seed maturation for the

acquisition of desiccation tolerance and dormancy. Arabidopsis plants carrying *abi3* mutant alleles, including *abi3-5*, yield seeds with strong physiological alterations. In a suppressor mutagenesis screen we obtained an *abi3-5* suppressor mutant, *suppressor of abi3-5* (*sua*), which reverted all of the *abi3-5* mutant phenotypes. Fine mapping and map based cloning revealed that *SUA* is a single copy, highly conserved gene that codes for a nuclear RNA binding protein. Analysis of *ABI3* transcripts showed that the *sua* mutation causes the splicing of a cryptic intron from the *ABI3* pre-mRNA. The novel splicing variant, in the mutant *abi3-5* background only, codes for a shorter but functional version of the ABI3 protein. In *sua abi3-5* double mutant seeds this protein could be detected with an immunological assay. A yeast two hybrid screen for SUA interactors identified a well characterized pre-spliceosomal complex component, the auxiliary factor of the U2 small nuclear ribonucleoprotein U2AF65. The SUA-U2AF65 interaction has been confirmed in Arabidopsis by FRET/FLIM.

We propose that SUA regulates *ABI3* alternative splicing by promoting the correct assembly of the pre-spliceosomal complex on *ABI3* pre-mRNA.

P470 Investigating uniparental expression of small RNAs involved in transposable element silencing in Arabidopsis

Amy Beeken

University of Cambridge, Department of Plant Science, Cambridge, UK

Transposable elements are mobile genetic elements that are highly abundant in plant genomes but are generally transcriptionally silenced to avoid the deleterious effect of transposition upon the genome. Transposable element silencing occurs due to RNA-dependent DNA methylation (RdDM), which involves RNA interference enzymes and Pol IV dependent small RNAs (p4-siRNAs) and results in non-CG methylation. Endogenous p4siRNAs are uniparentally expressed and abundant in the endosperm shortly after fertilisation (Mosher *et al*, 2009). The long terminal repeat (LTR) Tnt1 tobacco retrotransposon was introduced into Arabidopsis and is silenced when a threshold number of copies of the Tnt1 element is reached. The silencing of Tnt1 is associated with non-CG methylation of its promoter and p4-siRNAs targeting the LTR regions (Perez-Hormaeche *et al*, 2008). Reciprocal crosses with Arabidopsis lines containing various numbers of copies of the Tnt element, lines additionally containing the GUS translational fusion (LTR-GUS), and a Pol IV mutant line have been performed and the resulting seeds (and siliques) have been analysed via northern analysis and GUS staining techniques. The results provide insight into the uniparental expression of small RNAs in the endosperm that are involved in the silencing of mobile genetic elements.

Mosher *et al*, 2009, Nature, In press.

Perez-Hormaeche *et al*, 2008, Plant Physiology, Vol. 147, pp.1264-1278

New Tools and Resources

P471 Developments in TILLING inaccessible regions in complex genomes using Pyrosequencing

David Baker1, Simon Margerison2, Darren Heavens1, Pauline Stephenson3, Lars Ostergaard3, Jonathan Clarke1

1John Innes Genome Lab, Norwich, UK, 2Qiagen Ltd, Crawley, UK, 3John Innes Centre, Norwich, UK

Targeting Induced Local Lesions in Genomes (TILLING) is a strategy used in reverse genetic studies to identify series of chemically induced point mutations in specific genes. The detection of point mutations exploits the ability of the CEL1 endonuclease to cleave genomic DNA at mis-matched hetero-duplexes. When target sequences are very repetitive many mis-matches are formed causing screening to be severely hampered. Organisms with complex genomes can withstand high doses of chemical mutagen EMS and maintain correspondingly high mutation loads. Targeting a much smaller region in these organisms, avoiding the repetitive sequences, it can be cost effective to utilise Pyrosequencing to target particular hotspots of amino acids. The sensitivity of Pyrosequencing allows the simultaneous detection and sequence confirmation of a pooled sample. Here we discuss the progress so far and assess the suitability of this method to be used as an alternative TILLING strategy in a high throughput TILLING laboratory.

P472 Targeted genome capture and massively parallel sequencing as a tool to identify sequence variations in *Arabidopsis thaliana*

Darren Heavens1, Ivan Baxter2, Ed Lobenhofer3, David Salt2, Jonathan Clarke1

1John Innes Genome Laboratory, Norwich, UK, 2Horticulture and Landscape Architecture. Purdue University, West Lafayette, Indiana, USA, 3Cogenics, A Division of Clinical Data, 100 Perimeter Parkway, Suite C

Marker assisted selection has been a useful tool to aid identification of chromosomal loci of economic interest in crops and livestock without the need to identify variations in the predisposing genes. With the recent advances in massively parallel sequencing coupled with genome capture techniques it is now possible to rapidly isolate and sequence targeted genomic regions to make identification of the gene specific variations economically viable.

Using *Arabidopsis thaliana* as a model we present the methods employed to design the genomic capture array using Agilents e-Array platform, the enrichment process and quality control measures applied and the subsequent Roche 454FLX sequencing and data analysis to distinguish sequence variants in targeted genomic regions previously identified through Bulk Segregant Analysis (BSA).

P473 Genes for seed quality: New tools and approaches

Ronny Joosen

Wageningen University, Netherlands

Seed performance is a very complex trait, which comprises a large number of physiological principles related to important plant developmental processes. We use a physiological genetical genomics approach to survey these processes and resolve the underlying molecular mechanisms.

We are using the Arabidopsis Bay-0xSha RIL population and are in the process of locating phenotypic QTLs for diverse seed quality traits, like the germination performance under various environmental stresses. To be able to generate cumulative germination curves in a high throughput manner, we have developed an automated germination scoring system, in which we combined sophisticated image analysis with newly developed curve fitting software.

In parallel, we perform a comprehensive eQTL study on different developmental seed stadia using a generalized setup (Li *et al*, 2008). For this eQTL study we use the SNPtile array, a new Affymetrix tiling array that also harbours 250K SNPs. The combination of this transcriptomic and SNP data with the genetical power of a comprehensively phenotyped RIL population will open a new and exciting area in the field of physiological genetical genomics.

Li, Y., Breitling, R. and Jansen, R.C. (2008) Generalizing genetical genomics: getting added value from environmental perturbation. Trends in Genetics. 24, 518-524.

P474 Mapping of the Arabidopsis ER and post-ER glycoproteome

Alexander van der Krol1,2,3, Remco Mentink1, Maurice Henquet 2,3,4, Aalt-Jan van Dijk2,4, Dirk Bosch4, Wei Song1, Jan Cordewener4, Twan America4

1Lab. of Plant Physiology, Wageningen University, Netherlands, 2Center for BioSystems and Genomics, 3Netherlands Proteomics Centre, 4PRI, Wageningen UR, Netherlands

The occupancy of N-glycosylation sites was mapped for leaf proteins isolated from Arabidopsis WT and the mutant *cgl*, which lacks the activity in the Golgi to modify 'mannose type' N-glycans to 'complex-type' N-glycans. Proteins were digested with trypsin and glycopeptides were selectively coupled to Hydrazide resin. Bound peptides were released by PNGaseF (release peptides bound by 'mannose' N-glycans but not those bound by complex N-glycans) or PNGAseA (releases all peptides bound by N-glycans).

Results will be shown for peptide identity and confirmation of N-glycosylation at different confidence levels, based on (1) specific binding to Hydrazide, (2) comparison of measured Mass to predicted Mass, (3) peptide fragmentation pattern (Mascot), (4) AA-sequence containing the consensus glycosylation site (Proteinlynx or Mascot), (5) confirmed N to D conversion resulting from the PNGase treatment and (6) literature.

We identified glycoproteins previously not recognized as secreted protein because they lack a clear ER import signal peptide. Several glycoproteins with multiple glycosylation sites show heterogeneous modifications on the same protein. The signal ratio of (glyco)peptide masses from WT and *cgl* samples varies for individual glycoproteins, suggesting individual differences in subcellular distribution over the secretory pathways. The glycopeptide mapping also provides clues for the membrane topology of membrane glycoproteins, some of which deviate from HMRR predicted topology.

The results show that selection of glycopeptides reduces the complexity of the MS signature sufficiently for direct LCMS analysis, allowing for HTP proteomics of this important subset of the Arabidopsis proteome.

P475 Plant Methods: An independent open access journal for technological innovation in the plant sciences

Brian Forde, Mike Roberts

Lancaster University, Lancaster, UK

Plant Methods was established in 2005 as an open access electronic journal specialising in the rapid publication of peer-reviewed articles with a focus on technological innovation in the plant sciences. Plant Methods is published by BioMed Central, but belongs to its large group of over 100 independent journals.

Supported by a prestigious international editorial board, the journal's primary aim is to stimulate the development and adoption of new and improved techniques and research tools in plant biology. We have now published over 80 papers describing new techniques or resources of value to the plant biology community. In addition to methodology papers, we publish research papers, reviews and commentaries as well as occasional 'protocol' papers providing step-by-step descriptions of previously established techniques.

The journal has recently been indexed by ISI Web of Science and will receive its first official Impact Factor in June 2010 (its current unofficial impact factor is 2.06). This recognition by ISI is a strong endorsement of the journal's success to date and reflects its growing prominence and reputation in the field. The open access format of the journal, combined with its indexing in ISI (as well as PubMed and PubMed Central) ensures the widest possible visibility and readership for its authors.

Note that researchers whose institutions are Members of BioMed Central can publish articles in Plant Methods without any direct cost to them, or at a discounted rate in the case of Supporter Members. To find out if your institution is a BMC member go to http://www.biomedcentral.com/inst/. There are no additional charges for colour figures.

We encourage everyone to visit the journal at www.plantmethods.com and invite potential authors to consider submitting their next methodology paper to Plant Methods. For further information, contact Brian Forde (Editor-in-Chief) or Mike Roberts (Deputy Editor) at plantmethods@lancaster.ac.uk.

P476 TAIR - The first ten years, and the next five

Eva Huala, Debbie Alexander, Tanya Berardini, Raymond Chetty, Anjo Chi, Kate Dreher, Margarita Garcia-Hernandez, A S Karthikeyan, Vanessa Kirkup, Philippe Lamesch, Cynthia Lee, Donghui Li, Tom Meyer, Robert Muller, Larry Ploetz, Leonore Reiser, Rajkumar Sasidharan, Shanker Singh, David Swarbreck, Chris Wilks, Peifen Zhang

Carnegie Institution for Science, Stanford, CA, USA

TAIR (http://arabidopsis.org) is a worldwide resource for Arabidopsis data and a leader in the field of biological data curation. TAIR's team of professional curators and software developers organizes, integrates, curates and provides access to the most complete body of experimental data and biological resources available for any plant species. Over the first 10 years of its existence TAIR has evolved into an indispensable resource for plant biologists. A summary of TAIR's growth and achievements to date will be presented, along with plans for further development in the next 5 years.

P477 CORNET: A user-friendly tool for data mining and integration

Stefanie De Bodt, Diana Carvajal, Jens Hollunder, Joost Van den Cruyce, Sara Movahedi, Dirk Inzé

VIB/UGent, Department of Plant Systems Biology, Gent, Belgium

An enormous amount of functional genomics data has been generated. However, the retrieval, integration, analysis and interpretation of these data remain cumbersome for many biologists. To enable the advance of (systems) biological research, these tasks need to be facilitated. For example, gathering and processing microarray data from public databases related to a particular biological process are not straightforward. Neither is the compilation of protein-protein interaction data from numerous, partially overlapping databases. In addition, countless tools for data mining and integration have been developed with their specific aims and limitations. We developed CORNET as an access point to transcriptome, protein interactome, localization data and functional information on Arabidopsis. We offer a flexible web tool that allows the investigation of public data in comparison to your personal data. Foremost, microarray data can be browsed using ontology terms, processed and downloaded and co-expression analysis on predefined or user-defined expression datasets can be performed. The following questions can be addressed: Which genes are coexpressed with my gene of interest?, Does my gene of interest reside in a highly coexpressed gene cluster, or Do my differentially expressed genes show similar expression in diverse microarray experiments? On top of that, both predicted and experimentally identified protein-protein interactions and localization data can be integrated. As such, one can investigate if interacting proteins are encoded by coexpressed genes and similar localization or if an identified protein-protein interaction was detected by other approaches. Finally, network representation and further exploration are made easy by making use of Cytoscape. Currently, we employ CORNET to decipher the molecular networks governing leaf organ size.

P478 A database of a large-scale mutant collection and phenotype analysis for nuclear-encoded chloroplast proteins

Fumiyoshi Myouga1, Kenji Akiyama1, Reiko Motohashi2, Takashi Kuromori1, Rie Ryusui1, Tetsuya Sakurai1, Kazuo Shinozaki1

1RIKEN Plant Science Center, Yokohama, Japan, 2Shizuoka University, Shizuoka, Japan

More than 10% of the entire nuclear genome of Arabidopsis have been assigned to code for chloroplast proteins. For the functional analysis of the nuclear-encoded chloroplast proteins, we systematically collected their knockout mutant lines in Arabidopsis. Based on databases of international seed stock centers, we selected 3,244 *Ds/Spm* transposon or T-DNA tagged-lines for genes encoding1,369 chloroplast proteins; about 66 % of 2,090 predicted chloroplast proteins (Richly and Leister, 2004). In this collection, we so far observed 1,536 tag-lines corresponding to 830 chloroplast proteins. We found 230 mutants corresponding to 158 genes (19%) showing either seedling phenotypes or embryo lethal phenotypes. In our collection, there are 64 genes of 158 genes with multiple mutant alleles that show nearly the same phenotype. There are 72 genes of 158 genes that are previously reported, and a part of their mutant phenotype data is also available from TAIR or NCBI. These results suggest that our mutant resource and phenotype data provides highly reliable linkages between the phenotypes and genotypes of nuclear-encoded chloroplast proteins. Our results are recently open and can be accessed at the web site http://rarge.psc.riken.jp/chloroplast/, that is a public database and analysis resource for the study of chloroplast-targeted proteins not only essential proteins but also proteins non-essential to plastid function. Relationships between genotypes and phenotypes are important for systematic approaches of functional genomics research in Arabidopsis. Our database provides comprehensive information to connect individual genes to plastid functions based on our tagged mutant collections.

P479 Development of SVM-based prediction algorithms for plant peroxisomal PTS1 proteins by a novel iterative approach integrating computational science and experimental cell biology

Esteban Antonicelli1, Thomas Lingner2,3, Amr Kataya1, Aline Benichou1, Peter Meinicke2, Sigrun Reumann1

1Centre for Organelle Research, Stavanger, Norway, 2Department of Bioinformatics, Goettingen, Germany, 3Centre for Genomic Regulation, Barcelona, Spain

To comprehensively understand organelle metabolism and signalling networks, organelle proteomes must be characterized in their full complexity. High accuracy

prediction tools, however, are currently lacking for plant peroxisomal matrix proteins. To generate a training dataset of high quality and quantity of plant PTS1 sequences, we established experimental proteomics methodology for Arabidopsis and identified a large number of novel proteins including low-abundance and regulatory proteins. Peroxisome targeting of many proteins by the PTS1 pathway was verified by *in vivo* subcellular targeting studies using GFP fusion proteins. A large training dataset (2500 PTS1 sequences) was generated from EST databases. We applied a discriminative machine learning approach and developed two different models: (1) position-specific weight matrices (PWM) and (2) residue interdependence models (RIM). After training model-specific classification thresholds were calculated and a prediction accuracy of >99% sensitivity and >98% specificity determined. Ambiguous novel PTS1 tripeptides and PTS1 domains were verified by *in vivo* subcellular targeting analysis and the computational prediction threshold was re-defined experimentally. The prediction models were applied to the Arabidopsis genome, and representative unknown proteins are tested for peroxisome targeting *in vivo*. In an unprecedented iterative approach among targeting prediction tools these positive candidates are re-subjected to the search for homologous ESTs, training dataset extension and model development to further improve the prediction models.

The predicted Arabidopsis PTS1 proteins will be published in the new relational database AraPerox 2.0, and a public prediction server is being developed.

P480 Transcriptome profiling of RNA-processing mutants – Uncovering the hidden layer of the Arabidopsis transcriptome

Sascha Laubinger1, Georg Zeller2, Stefan R Henz1, Timo Sachsenberg1, Gunnar Rãtsch2, Detlef Weigel1

1Max Planck Institute for Developmental Biology, Tuebingen, Germany, 2Friedrich Miescher Laboratory, Tuebingen, Germany

Many recent publications report about the pervasive transcription of almost the entire genome of several different model organisms. In the model plant *Arabidopsis thaliana*, 32,000 protein-coding genes, pseudogenes and non-coding RNAs are annotated and this number is still expected to rise. We utilized whole genome tiling arrays and found more then 1,000 'intergenic' regions that are actively transcribed during the life cycle of wild-type plants as well as novel transcripts that occur only under certain stress conditions such as salt, heat or cold stress. In addition, analyses of mutants impaired in miRNA processing, splicing, or mRNA quality control reveal the existence of many transcripts that are undetectable in wild-type plants such as transcripts that are adjacent to silenced transposons. Such RNAs might constitute unstable, but important regulatory molecules of the Arabidopsis transcriptome, RNA processing intermediates that are eliminated during RNA maturation, or simply ghosts of past evolution.

We will discuss the identification of previously unknown RNAs and potential new RNA maturation pathways, and conclude by giving an outlook on RNA-seq applications.

P481 Molecular tools for studying the Arabidopsis transcription factor ORFeome

Christoph Weiste1, Tim Iven1, Nora Glaser1, Stefan Dröge1, Andrea Hermann1, Luis Oñate-Sánchez2, Wolfgang Dröge-Laser1

1Dep. Biotecnologia, Albrecht-von-Haller-Institut, University of Göttingen, Untere Karspüle2, D-37073 Göttingen, Germany, (wdroege@gwdg.de); 2Centro de Biotecnologia y Genomica de Plantas (UPM-INIA). Campus de Montegancedo, Autopista M-40 (Km 38), Universidad Politecnica de Madrid.28223-Pozuelo de Alarcon (Madrid) Spain

Genomic approaches have generated large Arabidopsis open reading frame (ORF) collections. However, tools are required to functionally characterise this ORFeome. This AFGN project aims to develop several technology platforms based on an existing transcription factor (TF) ORFeome consisting of 1200 full-length GATEWAY® compatible cDNA clones. (1) We have developed a novel batch procedure to simultaneously recombine GATEWAY®-tagged cDNAs into a plant expression vector. This pool has been applied to build up an *Arabidopsis thaliana* TF ORF over expressing (AtTORF-Ex) seed stock library[1] over expressing more than 250 independent TF genes covering AP2/ERF, bZIP and WRKY TFs. We demonstrate screening procedures to define TF function in development and stress defense. A complementary approach making use of repressor domain fusions has been demonstrated and used for loss-of-function screens. These molecular tools will significantly improve functional studies on TFs performed by the Arabidopsis community.

1 Weiste C, Iven T, Fischer U, Oñate-Sánchez L, Dröge-Laser W. (2007) *In planta* ORFeome analysis by large-scale overexpression of GATEWAY®-compatible cDNA clones - screening for transcription factors involved in stress defense. Plant J. 52: 382-390.

P482 Resources for Gateway recombinational cloning in plant cells

Mansour Karimi, Rebecca De Clercq, Björn De Meyer, Rudy Vanderhaegen, Wilson Ardilez, Pierre Hilson

Dept. Plant Systems Biology, VIB - Ghent University, Belgium

The Gateway technology (www.invitrogen.com) has been developed to facilitate the transfer of DNA segments between plasmids by site-specific recombinational cloning. We have constructed a large collection of Gateway-compatible destination vectors for a wide range of gene function analyses in transgenic plant cells. Using MultiSite recombination Gateway cassettes, plant binary destination vectors have also been created in which two or three segments can be transferred contiguously or in independent expression units, in a single LR clonase *in vitro* reaction. Our destination vectors carry one of three plant selectable markers coding for resistance to kanamycin (*nptII*), hygromycin (*hpt*) or glufosinate ammonium (bar), and are available in small high copy number plasmids.

To further streamline the construction of recombinant genes, we have built a series of reference Gateway entry clones carrying promoters, terminators, and reporter open

reading frames most commonly used in plant research. This collection obeys simple engineering rules: the genetic elements (parts) are designed in a standard format; they are interchangeable, fully documented, and can be combined at will according to the desired output.

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

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

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

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

P483 AraCyc and PlantCyc: Metabolic pathway databases for plant research

K Dreher, S Singh, M Ahn, A Chi, K He, E Huala, A S Karthikeyan, V Kirkup, C Lee, R Leitao, T Meyer, R Muller, L Ploetz, S Rhee, S Singh, C Wilks, P Zhang

Carnegie Institution for Science, Stanford, CA, USA

AraCyc and PlantCyc, two metabolic pathway databases, house information about metabolic genes, enzymes, compounds, reactions, and pathways present in *Arabidopsis thaliana* and many other plants (www.plantcyc.org). In March 2009, new releases of AraCyc (5.0) and PlantCyc (2.0) debuted at TAIR and the Plant Metabolic Network. Each updated database contains new information and also includes substantial revisions to a number of pathways and enzymatic reactions present in the previous releases. In AraCyc, efforts focused on a) reviewing over 1700 enzyme/reaction assignments, primarily based on published literature and b) incorporating new sets of compounds identified through experimentation. In PlantCyc, the data content grew considerably as large sets of orthologous enzymes from *Medicago truncatula*, rice, and tomato were incorporated into many pathways. In both databases, pathways were added or updated to reflect the growing body of knowledge concerning plant metabolism. AraCyc and PlantCyc can help enhance the research efforts of biochemists and non-biochemists alike. For instance, genetic screens often identify genes involved in metabolism, and, with these databases, researchers can learn about the biochemical pathways and other enzymes related to their gene of interest in plants. In addition, the OMICs viewer data display tool in AraCyc enables researchers to examine the results of their microarray, proteomic, or metabolomic experiments in a metabolic context. Pathways that are up-regulated or down-regulated can be quickly visualized in a metabolic context, even in plants that may have no obvious phenotypic abnormalities, allowing researchers to generate testable hypotheses for further study. To make these resources even more useful, work is underway on the next versions of the databases. New data from actively researched domains of metabolism and new tools for analysis are starting to be incorporated into these continually improving databases.

P484 Imaging of individual leaf expansion in three dimensions: The analysis of cell volume of epidermal and sub-epidermal tissues

Nathalie Wuyts1, Genevieve Conejero2, Jean-Luc Verdeil2, Catherine Massonnet1, Jean-Luc Regnard3, Evelyne Costes3, Christine Granier1

1Lepse, IBIP, INRA-SupAgro, Montpellier, France, 2PhiV, UMR DAP, CIRAD, Montpellier, France, 3AFEF, UMR DAP, INRA-SupAgro, Montpellier, France

As most leaf development studies and phenotyping efforts only take the leaf surface into consideration, little is known on the establishment of leaf volume and the interaction between different cell layers (epidermal and sub-epidermal), or on the effects of genetic alterations and environmental perturbations on leaf volume. A project has therefore been initiated to develop a method for high resolution imaging and analysis of leaf tissue in three dimensions, concentrating in first instance on developing leaves of *Arabidopsis thaliana* and apple tree, a model and an agronomic species, respectively. The method includes a tissue preparation phase for clearing and coloration, an imaging phase using multiphoton laser scanning microscopy for optical sectioning, and an image analysis phase for volume measurement. Macros have been written in ImageJ, an open-source image analysis environment, for the collection of data on leaf thickness, volumetric proportions of tissues, and cell densities and volumes in different tissues with an estimation of airspaces in sub-epidermal tissues. The method will be of use in complementing our knowledge of individual leaf growth processes, extending the identification of leaf phenotypes of genetically or environmentally disturbed plants and providing a structural basis for the analysis of leaf function.

P485 Fluorescence activated cell sorting (FACS) of embryonic cells by use of the *DRN::erGFP* marker

Melanie Cole, Wolfgang Werr

Department of Developmental Biology, University of Cologne, Germany

Transcriptional profiling of genes expressed early during embryogenesis is limited by the availability of embryonic tissue. The AP2 transcription factor *DORNRÖSCHEN (DRN)* is activated in the apical cell after the first zygotic division and is expressed uniformly in the apical cell lineage until the globular stage, when transcription gets confined to the emerging cotyledons. We have raised embryogenic suspension cultures of bending stage embryos of transgenic *DRN::erGFP* lines. GFP activity is recovered on the surface of suspension calli and *DRN::erGFP*-positive cells can easily be separated from non expressing cells via FACS sorting of protoplasts. RNA isolated from GFP-positive and -negative cells will be

subjected to microarray analysis and the comparison will identify potential target genes of the *DRN* AP2-type transcription factor. We will present real-time PCR data, which will reveal whether sorted *DRN::erGFP* positive protoplasts have acquired an early embryonic cell fate. These data will resolve the question whether somatic embryogenesis starts with reprogramming of embryonic cell fate or with resetting of callus cells to a meristematic potential.

P486 New data, new challenges – Improving Arabidopsis annotation

David Swarbreck, Philippe Lamesch, Cynthia Lee, Robert Muller, Rajkumar Sasidharan, Chris Wilks, Eva Huala

Carnegie Institution for Science, Stanford, CA, USA

It is nine years since the genome sequence of A.thaliana was 'completed'. In the intervening period the annotation has undergone significant refinement with many updates to the exon-intron structures as well as the addition of more than 2000 new genes. A new TAIR genome release (TAIR9) was published earlier this year that saw the addition of 282 novel loci and updates to 1254 gene models. Numerous plant genome sequencing projects are currently in progress. *A. thaliana* as the foremost model organism in plant biology will provide an important reference when determining comparative genomic approaches and annotation practices. As such a gold standard of annotation is demanded for the Arabidopsis genome. Traditionally genome annotation has been hindered by lack of transcript data. However, technological advances have facilitated large scale transcriptome sequencing projects, and allowed for extensive sampling of the Arabidopsis proteome via tandem mass spectrometry.

While such data is hugely beneficial it also presents its own unique challenge. TAIR will present details of the latest genome release, describe its process of re-annotation, and discuss the complexities of utilising large scale transcriptome and proteomic data for improving annotation.

P487 The CPIB Root Atlas: A tool for viewing integrative root systems biology data

Michael Wilson, Tara Holman, Andrew French, Mike Stout, Darren Wells, Kim Kenobi, Malcolm Bennett, Mike Holdsworth, Tony Pridmore, T Charlie Hodgman

The Centre for Plant Integrative Biology, University of Nottingham, UK

The Centre for Plant Integrative Biology (CPIB) at the University of Nottingham integrates biologists, bioinformaticians, computer scientists, engineers and mathematicians towards generating a virtual model of root growth and development.

To this end, CPIB are in the process of generating a large amount of dynamic multi-scale high-resolution datasets relating to anisotropic root expansion. These datasets will consist of transcriptomic, glycomic, image, model and other data at different locations and developmental points, and cover wild-type, various mutants and different treatment conditions.

One of CPIB's biggest challenges is the integration of these disparate data types into a model of the Arabidopsis root. As part of the solution, we plan the creation of the *Arabidopsis thaliana* localisome (Atlas) viewer, which will integrate the data into a human intelligible and navigable resource.

I will describe current progress on the development of the CPIB transcriptomics repository, a web accessible resource allowing access to CPIB's primary data sets and knowledge resources as well as partially guided investigatory views over the data.

A key component is the integrative Atlas viewer, an interactive catalogue of CPIB's data sets and the front end to the web-resource, allowing both secure and public access to CPIB's resources for all spatio-temporal views within the projects remit.

P488 SUBA: The Arabidopsis protein subcellular database

Sandra K Tanz1, Joshua L Heazlewood1, Julian Tonti-Filippini1,2, A Harvey Millar1,2, Ian Small1,2

1ARC Centre of Excellence in Plant Energy Biology, UWA, Australia, 2Centre of Excellence in Computational Systems Biology, UWA, Australia

Information on the subcellular location of proteins makes an important contribution towards our understanding of protein function and of biological inter-relationships. A variety of medium- to high-throughput technologies are currently employed to determine the subcellular location of proteins. Each year since 2005, we have been bringing together new data on a wide range of data sources to build SUBA (*SUB*cellular location database for Arabidopsis proteins) comprising large-scale proteomic and GFP localisation datasets for different subcellular compartments of Arabidopsis.

Currently, 2232 entries in SUBA are based on fluorescent protein chimeric fusion studies (1500 distinct proteins) and 9603 entries are based on subcellular proteomic studies (4636 distinct proteins). SUBA also contains precompiled bioinformatic predictions for protein subcellular localization, including TargetP, Predotar and iPSORT. The database has a web interface that allows advanced combinatorial queries to be undertaken on the contained data. Photographic images of fluorescent protein localizations are now being added to SUBA to allow users direct interpretation of complex patterns alongside prediction and proteomic data. SUBA provides a powerful tool and a one-stop-shop for defining protein location in Arabidopsis cells.

Analysis of the data provides information on gold-standard single location proteins that can be used as subcellular markers, highlights conflicts between published datasets, identifies potentially multi-targeted proteins and allows false positive rates for subcellular location tools to be assessed. SUBA was updated in March 2009 and is based on the TAIR8 genome annotation release.

P489 Alternative splicing and NMD in Arabidopsis

Craig Simpson1, Maria Kalyna2, Dominika Lewandowska1, Branislav Kusenda2, John Fuller1, Linda Milne1, Jim McNicoll3, Gillian Clark1, Andrea Barta2, John Brown1,4

1Scottish Crop Research Institute, Dundee, UK, 2Max F Perutz Laboratories, Vienna, Austria, 3BioSS, Scottish Crop Research Institute, Dundee, UK, 4University of Dundee@SCRI, Dundee, UK

We have established an RT-PCR panel to monitor changes in alternatively spliced isoforms of ca. 300 Arabidopsis alternative splicing events. The events were selected from publications and databases with a focus on events in transcription factors, RNA-interacting proteins and proteins involved in signalling or stress responses. The relative abundance of different isoforms are calculated from three biological reps and compared between treatments. In Arabidopsis, functional links between AS and NMD have been shown for some genes such as the genes encoding circadian clock proteins, *AtGRP7* and *AtGRP8*.[1,2] To examine the wider relationship between alternative splicing and NMD, we have analysed total RNA from wild-type plants and mutants of the NMD proteins, UPF1 and UPF3. The *upf3-1* and *upf1-5* are viable mutants impaired in NMD. The alternative splicing profiles were also compared to plants treated with cycloheximide to block translation and thereby NMD. Impaired NMD will lead to an increase in abundance of RT-PCR products of alternatively spliced isoforms, which are normally turned over by NMD. The 264 alternative splicing events analysed produced around 700 alternatively spliced transcripts of which approximately 150 increased in abundance. A number of the NMD-sensitive RT-PCR products were unknown and are being sequenced to confirm that they are isoforms and identify the presence and position of premature termination codons. We estimate from these results that between 15-20% of alternatively spliced products in Arabidopsis are turned over by NMD.

1 Schöning *et al* (2007) Plant J. 52: 1119-1130;
2 Schöning *et al* (2008) Nucleic Acids Res. 36: 6977-6987.

P490 Mining small RNA sequencing data: A new approach to identify small nucleolar RNAs in Arabidopsis

Ho-Ming Chen, Shu-Hsing Wu

Institute of Plant and Microbial Biology, Academia Sinica, Taipei, Taiwan

Small nucleolar RNAs (snoRNAs) are non-coding RNAs that direct 2'-O-methylation or pseudouridylation on ribosomal RNAs or spliceosomal small nuclear RNAs. These modifications are needed to modulate the activity of ribosomes and spliceosomes. A comprehensive repertoire of snoRNAs is needed to expand the knowledge of these modifications. The sequences corresponding to snoRNAs in 18 to 26-nt small RNA sequencing data have been rarely explored and remain as a hidden treasure for snoRNA annotation. Here we showed the enrichment of small RNAs at Arabidopsis snoRNA termini and developed a computational approach to identify snoRNAs on the basis of this characteristic. The approach successfully uncovered the full-length sequences of 144 known Arabidopsis snoRNA genes, including some snoRNAs with improved 5'- or 3'-end annotation. In addition, we identified 27 and 17 candidates for novel box C/D and box H/ACA snoRNAs, respectively. Northern blot analysis and sequencing data from parallel analysis of RNA ends confirmed the expression and the termini of the newly predicted snoRNAs. Our study especially expanded on the current knowledge of box H/ACA snoRNAs and snoRNA species targeting snRNAs. In this study, we demonstrated that the use of small RNA sequencing data can increase the complexity and the accuracy of snoRNA annotation.

P491 The classical genetic map of *Arabidopsis thaliana*

David Meinke

Oklahoma State University, Stillwater, OK, USA

In recognition of the 20th International Conference on Arabidopsis Research, I describe here the current status of the classical genetic map of Arabidopsis, which was first published 26 years ago by Koornneef *et al* (J. Heredity, 74: 265-272). The updated map includes 340 loci associated with a defined mutant phenotype, a significant increase beyond the 76 markers included on the original map. Consistent with the working definition of a classical genetic map, the current dataset is limited to mutant genes mapped using recombination data obtained with other visible markers. Genes localized only in relation to molecular or physical markers are for the most part excluded. Forty percent of the genes included on the classical map exhibit a seed phenotype. Seventy percent of these mapped *EMB* genes remain to be cloned. Recent progress towards identifying these genes is described in a separate poster from my laboratory. Almost 90% of mapped visible markers with other mutant phenotypes have been cloned. An updated list of mapped and cloned genes is presented here. Ultimately, the classical map needs to be replaced by a comprehensive, sequence-based map of cloned Arabidopsis genes associated with a mutant phenotype. The first version of such a map was published 6 years ago (Meinke *et al*, Plant Physiol., 131: 409-418). My laboratory is currently updating this dataset based on information obtained from TAIR and the published literature. Eventually, we hope to incorporate the list of cloned genes with a loss-of-function mutant phenotype into TAIR so that it can be widely used, thoroughly checked, and regularly updated by members of the community.

P492 Characterization of fluorescent carbohydrate binding dyes for analysis of the cell wall

Andrew Carroll1,2, Charlie Anderson1, Laila Akhmetova3, Chris Somerville1,4

1Energy Biosciences Institute, Berkeley, California, USA, 2Stanford University, Stanford, California, USA, 3Middle Eastern Technical University, Ankara, Turkey, 4University of California, Berkeley, California, USA

The use of fluorescent fusion proteins has become an essential tool for the analysis of protein function and

localization. Obtaining similar information about the localization and function of carbohydrates in the plant cell wall has proven problematic due to the relative difficulty of labeling specific wall components. Here we present the characterization of two fluorescent, carbohydrate-binding dyes, Solophenyl Flavine 7GFE and Pontamine Scarlet S4B for the analysis of cell walls. 7GFE was found to label xyloglucan and cellulose, while S4B was found to label cellulose more specifically. Confocal microscopy on labeled root tissue revealed a fibrillar pattern of cellulose in the cell wall which transitioned from transverse near the membrane to longitudinal at the outer wall. Timelapse microscopy revealed that the cellulose fibrils reoriented toward a more longitudinal orientation over time. These results support a model of the cell wall in which anisotropy is generated in part by the dynamic reorientation of cellulose within the cell wall. They also suggest that the family of direct dyes, which contains hundreds of members, may prove useful for the visualization and analysis of other cell wall components.

P493 RARGE: A web database of Arabidopsis resources

Kenji Akiyama, Fumiyoshi Myouga, Takashi Kuromori, Kazuo Shinozaki, Tetsuya Sakurai

RIKEN Plant Science Center, Yokohama, Japan

RIKEN Arabidopsis Genome Encyclopedia (http://rarge.psc.riken.jp/), commonly known as RARGE—is a web database that provides basic information about the Arabidopsis genome, such as that related to cDNA sequences and transposon-insertion mutants. Some contents of this database, which has been available since 2003, have been improved in the past few years on the basis of the results of sequence analyses. We have developed 2 datasets, RAPID (RIKEN Arabidopsis Phenome Information Database) and ChloroBase, based on the traits of plants with disrupted genes. RAPID is based on the results of a comprehensive observational study on Ds-transposon insertion mutants. The identification (ID) numbers of mutant lines and loci can be searched in the RAPID dataset, and detailed information, including photographs, can be browsed. ChloroBase is based on the results of a study on transposon and T-DNA-insertion mutants with disrupted nuclear-encoded chloroplast proteins. It contains data such as germination ratios and photographs. Although both these datasets are available on individual websites, we integrated the 2 databases into RARGE in order to improve their user friendliness.

P494 No bridge too far: A simple method for multi-protein interaction analysis

Christopher Grefen1, Annegret Honsbein1, Klaus Harter2, Mike Blatt1

1FBLS Plant Sciences - University of Glasgow, 2ZMBP Plant Physiology - University of Tuebingen

Protein-protein interactions are a prerequisite for many vital functions and their regulation in living cells. Methods for detecting these interactions *in vivo* frequently depend on heterologous co-expression of the potential interactors. Among these, the well-established Split-Ubiquitin System (SUS) in yeast allows analysis of full-length and membrane-bound proteins. However, like most other methods, SUS assays are not designed to test for multi-protein interactions, especially those which depend on a third component for complex formation.

Here we provide a method - the SUS-Bridge Assay (SBA) - based on the SUS assay which enables direct analysis of multi-protein interactions and their requirements for bridging partners in the binding dynamics of the complex. The SBA can be used to verify putative interaction complexes as well as screening for novel interactions in libraries of putative partner proteins.

P495 Summary of resource projects in RIKEN BRC

Shiori Ota, Hiroshi Abe, Satoshi Luchi, Toshihiro Kobayashi, Masatomo Kobayashi

RIKEN BRC, Tsukuba, Ibaraki 305-0074, Japan

RIKEN BioResource Center (BRC) preserves and distributes plant materials including RIKEN Arabidopsis transposon-tagged (RATM) line seeds and RIKEN Arabidopsis full-length cDNA (RAFL) clones etc., in accordance with the National Bio-Resource Project (NBRP) implemented by the Ministry of Education, Culture, Sports, Science and Technology of Japan. Since its establishment in 2001, BRC has been distributing approx. 33,000 materials to 1,200 laboratories over the world. There have been more than 200 manuscripts in which our materials were used for their researches.

In this spring, BRC made an announcement of expanding its distribution area of Arabidopsis T87 cultured cells to the research communities abroad. Currently the material is shipped by asking users to take responsibilities for the customs procedure at the time of shipment and payment of FedEx freight charge. And besides, RIKEN Arabidopsis full-length cDNA OvereXpressing gene hunting system (FOX line) seeds, developed by RIKEN Plant Science Center (PSC) are now available for screening. This resource is provided as a seed pool set. Seeds for individual lines that constitute specific seed pools are also available for the users who obtained a desired mutant in the seed pool. Other than Arabidopsis resources, RIKEN BRC distributes model plant resources such as full length cDNA of *Physcomitrella patens*, poplar and cassava and Tobacco EST clones from BY-2 cells. New and additional release of clones and seeds will be announced accordingly during the year of 2009. Further information is available from the BRC website http://www.brc.riken.go.p/lab/Eng/ or e-mail to plant@brc.riken.jp

P496 Expression analysis of short open reading frames (sORFs) in Arabidopsis by custom microarray

Mieko Higuchi, Kousuke Hanada, Youichi Kondou, Tatsuya Sakai, Yoko Horii, Mika Kawashima, Minami Matsui

RIKEN PSC, Yokohama, Japan

An open reading frame predicted to encode more than 100 amino acids is annotated as a gene. Therefore, the

initial annotation did not include many short open reading frames (sORFs). Recent transcriptome studies have discovered that small nonannotated protein-coding genes were expressed in intergenic regions. Recently, the peptides encoded by sORFs are reported to be involved in various functions in yeast and Drosophila. We identified 7,159 sORFs ranging from 90 to 300 bp in the intergenic regions of the Arabidopsis genome (Hanada *et al* 2007). To detect sORF expression in Arabidopsis, we developed a custom microarray including 5,921 sORFs, 26,192 AGI genes and 1,443 intergenic transcripts. Microarray analysis was conducted using RNA isolated from fully expanded rosette leaves. Expression of AGI genes and intergenic transcripts were detected in about 70% and 56% of total spot number, respectively. In contrast, expression of only about 9% sORF was detected in rosette leaves. Some sORF expressions were confirmed by RT-PCR analysis. RT-PCR analysis was also performed in various tissues (Leaf, Flower, Silique and Root). We found that some sORFs showed tissue specific expression patterns. Next, we focused on light-regulated expression change of sORFs. Seedlings grown in the dark for 3 days were transferred to continuous illumination. Seedlings were collected after 1, 6, 24 h illumination and extracted RNA was subjected to microarray analysis. Around 50 sORFs were repressed and 30 sORFs were induced after 24h illumination. These results suggest that sORF might have a role in light-regulated development in Arabidopsis.

P497 Where to fasten – transcription factors and their DNA-motif companions

Luise H Brand, Sabine Hummel, Achim Hahn, Klaus Harter, Dierk Wanke

ZMBP Plant Physiology, Tuebingen, Germany

Transcription factors are proteins that specifically bind to DNA and thereby govern the expression of their gene targets. The short conserved DNA motifs serve as interfaces that facilitate the integration of environmental and developmental stimuli into gene expression changes, thus realizing specific responses. However, only ~10 % of the transcription factors encoded by the eukaryote genomes possess known *cis*-regulatory DNA elements. This emphasizes the necessity to gain more detailed insight into this protein-DNA interaction. Therefore, we employed an ELISA-based method to study the *in vitro* binding specificities of some *Arabidopsis thaliana* transcription factors. We used crude extracts of recombinant protein to elucidate the binding capacities via immunological detection with the alcalic phospatase coupled enzyme reaction. This allows us to examine the specific qualitative and quantitative binding properties of the transcription factors and their DNA-motif companions under investigation. Eventually, we were able to confirm known and discover new previously unknown DNA motifs as transcription factor binding sites. To conclude, our results indicate the capability of this low priced method to refine contemporary knowledge on plant transcription factor DNA-binding relations.

P498 Optical contrast enhancement by decay shape analysis of fluorescence intensity decay curves

Frank Schleifenbaum1, Kirstin Elgass2, Marcus Sackrow2, Katharina Caesar1, Kenneth Berendzen1, Klaus Harter1, Alfred J Meixner2

1Center for Plant Molecular Biology, Department of Plant Physiology, University of Tübingen, 72076 Tübingen, Germany, 2Institute of Physical and Theoretical Chemistry, University of Tübingen, 72076 Tübingen, Germany

Background fluorescence deriving from subcellular compartments is a major drawback in high-resolution imaging of cells. We present a novel technique for contrast enhancement of a fluorescence image of over one order of magnitude. The method utilizes the shape characteristics of the fluorescence lifetime curve to determine undesignated background emission. Our approach enables the investigation of samples with high autofluorescence background, such as living plant cells, at high spatial resolution. We investigated *Arabidopsis thaliana* seedling cells in their tissue environment, which express an eGFP fusion of the plasma membrane-bound marker protein LTI6b[1] to demonstrate the feasibility of our method.

Cutler *et al* (2000), Proc. Natl. Acad. Sci. USA 97, 3718-3723.

P499 The Arabidopsis Biological Resource Center – Stock donation and distribution

Randy Scholl, Luz Rivero, Emma Knee, Deborah Crist, Christopher Calhoun, Natalie Case, Juan Castro, James Mann, Julie Miller, Bhuma Muthuvel, Garret Posey, Ridhima Shah, Pamela Vivian, Damitha Wickramasinghe, Hehua Yan, Zhen Zhang

The Ohio State University, Columbus, (OH), USA

The Arabidopsis Biological Resource Center was established at The Ohio State University in September, 1991. Our mission is to acquire, preserve and distribute seed and DNA resources that are useful to the research community. ABRC database functions and ordering system are incorporated into TAIR (http://arabidopsis.org). Researchers can obtain information about Arabidopsis, perform stock searches, order stocks, view orders and invoices, and make payments. New seed and DNA stocks enter ABRC through donation by national and international researchers. Donors from 27 countries have contributed to our holdings, and all donations are exchanged with the European Arabidopsis Stock Centre (NASC). 80% of our stocks have been donated by researchers in the USA. The majority of the remaining stocks were received from the United Kingdom, Germany, Belgium and Japan. Our current focus is expanding our collection of characterized mutants. We are promoting donation of published mutants and characterized, confirmed insertion lines (published and unpublished). We have developed a simplified donation form, which will aid donors in transmitting their stocks. We welcome donations of all types of seed and DNA resources. Donation forms for different classes of stocks are available online (http://abrc.osu.edu).

Distribution rates of seed and DNA stocks continue to be very strong. During the past year, ABRC distributed over 90,000 seed and DNA stocks to laboratories in 60 countries. 76% of seed stocks distributed are T-DNA insertion lines. Full-length / ORF cDNA clones represent 48% of the DNA stocks being shipped.

ABRC is supported by the National Science Foundation.

P500 Subcellular compartmentation of primary metabolism in *Arabidopsis thaliana* leaves – new insights from non-aqueous fractionation

Daniel Vosloh, Stéphanie Arrivault, Regina Feil, John Lunn, Mark Stitt

Max Planck Institute of Molecular Plant Physiology, 14476 Potsdam-Golm, Germany

Metabolism in plant cells is highly compartmented, with many pathways involving reactions in more than one compartment. For example, during photosynthesis in leaf mesophyll cells, primary carbon fixation and starch synthesis take place in the chloroplast, whereas sucrose is synthesized in the cytosol and stored in the vacuole. Classical fractionation methods such as sucrose density gradient centrifugation can be used for enzyme localization, but the separation of organelles by these methods is generally too slow compared to the turnover times of pathway intermediates to provide useful information about the distribution of metabolites. To circumvent this problem we are applying a technique, which fractionates the cells under non-aqueous conditions, whereby the metabolic state is frozen at the time of harvest and held in stasis throughout the fractionation procedure. Highly sensitive robotized cycling assays enable us to measure marker enzymes in the various cell fractions, along with about 20 enzymes and several metabolites (e.g. hexose-phosphates) from central metabolism. Application of liquid chromatography linked to tandem mass spectrometry (LC-MS/MS) has considerably extended the range of metabolites, especially phosphorylated intermediates, that can be measured in the cell fractions. With the combination of non-aqueous fractionation and LC-MS/MS, we can now investigate the intracellular distributions of most of the intermediates of photosynthetic carbon metabolism. We are especially interested in resolving the disputed pathway of starch synthesis in leaves by localizing ADP-glucose in wild type and mutant *Arabidopsis thaliana* plants, and in understanding the role of the signal metabolite trehalose 6-phosphate in the interaction of sucrose and starch metabolism.

P501 Streamlined sub-protocols for floral-dip transformation and selection of transformants

Amanda M Davis1, Anthony Hall2, Andrew Millar3, Chiarina Darrah1, Seth J Davis1

1Max Planck Institute for Plant Breeding Research, Cologne, Germany, 2University of Liverpool, Liverpool, UK, 3University of Edinburgh, Edinburgh, UK

Generating and identifying transformants is essential for many studies of gene function. In *Arabidopsis thaliana*, a revolutionary protocol termed floral dip is now the most widely used transformation method. Although robust, it involves a number of relatively time-consuming and laborious steps, including manipulating an *Agrobacterium tumefaciens* culture and aseptic procedures for the selection of plant lines harboring antibiotic-selection markers.

Furthermore, where multiple transgenes are to be introduced, achieving this by sequential transformations over multiple generations adds significantly to the time required. To circumvent these bottlenecks, we have developed three streamlined sub-protocols. First, we find that *A. thaliana* can be transformed by dipping directly into an *A. tumefaciens* culture supplemented with surfactant, eliminating the need for media exchange to a buffered solution. Next, we illustrate that *A. thaliana* lines possessing a double-transformation event can be readily generated by simply by floral-dipping into a mixture of two *A. tumefaciens* cultures harboring distinct transformation vectors. Finally, we report an alternative method of transformant selection on chromatography sand that does not require surface sterilization of seeds. These sub-protocols, which can be used separately or in combination, save time and money, and reduce the possibility of contamination.

See: http://www.plantmethods.com/content/5/1/3

P502 Characterising the mechanical properties of elongating root cells

Darren M Wells1,2, Rosemary J Dyson1,3, Ming Qian1, Malcolm Bennett1, T Charlie Hodgman1, Oliver E Jensen3, Nicola M Everitt2

1The Centre for Plant Integrative Biology, University of Nottingham, UK, 2Materials, Mechanics and Structures Division, Faculty of Engineering, University of Notingham, UK, 3School of Mathematical Sciences, University of Nottingham, UK

The Centre for Plant Integrative Biology (CPIB) at the University of Nottingham brings together biologists, engineers, mathematicians, bioinformaticians and computer scientists to generate new data, biological resources and multi-scale virtual models that will aid understanding of root growth and development.

Plant root cells elongate anisotropically with deposition of new material and modification of cell wall properties occurring over the entire wall (with the exception of root hairs which exhibit tip growth). This anisotropic growth pattern is dependent on the directional mechanical properties of the cell wall, conferred by a tightly regulated network of cellulose microfibrils embedded in the wall matrix.

CPIB is developing tools to characterise the mechanical properties of elongating root cells including compositional analyses, measurement and manipulation of turgor and microscopy techniques (atomic force, laser scanning confocal, and electron microscopy). This experimental work is used to inform mechanical models of the cell wall. Mechanical anisotropy effects are built into strain energy formulations and solved using finite element methods to investigate short timescale elastic deformations. They are also incorporated into transversely isotropic viscous fluid models, solved using asymptotic methods exploiting the geometry of the cell, which are used to model growth at longer timescales.

Experimental analyses of the structure and properties of the elongating cell wall and the mechanisms whereby walls are modified will be presented, together with techniques to parameterise current models for the control of cell expansion in roots.

P503 PosMed-plus: An information system making the utmost use of Arabidopsis knowledge to assist molecular breeding of crops

Yuko Makita1, Norio Kobayashi1, Kei Iida1, Satomi Asano1, Yuko Yoshida1, Naohiko Heida1, Mrinalini Deshpande1, Rinki Bhatia1, Akihiro Matsushima1, Manabu Ishii1, Yoshiki Mochizuki1, Kousuke Hanada2, Shuji Kawaguchi1, Takashi Kuromori2, Motoaki Seki2, Kazuo Shinozaki2, Tetsuro Toyoda1

1Bioinformatics And Systems Engineering (BASE) division, RIKEN. Yokohama, Japan., 2Plant Science Center (PSC), RIKEN. Yokohama, Japan.

Molecular breeding of agricultural crops such as rice is an efficient way to upgrade the plant functions useful to mankind. Forward genetics or positional cloning to identify the genes that confer useful functions to plants is a key step in molecular breeding. In order to accelerate the whole research process, we have developed an integrated database system powered by an intelligent data-retrieval engine termed PosMed-plus (Positional Medline for plant-updating science), allowing us to prioritize highly promising candidate genes in a given chromosomal interval(s). The system associates a user's query, such as phenotypic or functional keywords, with the genes existing within the interval by fully utilizing the integrated information of genome, transcriptome, proteome, localizome, phenome, and literature databases. The system supplies both a powerful integrative search function and a rich integrative display function of the integrated databases. PosMed-plus is the first information tool to prioritize candidate genes for forward genetics approaches in plant science and will be expanded to a wide use in plant-upgrading sciences in many plant species.

P504 Rice Fox Database: A database of rice full length cDNA overexpressed Arabidopsis mutants

Tetsuya Sakurai1, Youichi Kondou1, Atsushi Kurotani1, Kenji Akiyama1, Hitoshi Sakakibara1, Miyako Kusano1, Takeshi Yoshizumi1, Mieko Higuchi1, Shinya Takahashi1, Takanari Ichikawa1, Hirofumi Kuroda1, Hideki Takahashi1, Masaki Mori2, Hiroshi Takatsuji2, Kenji Oda 3, Hirohiko Hirochika2, Minami Matsui1

1RIKEN Plant Science Center, Japan, 2National Institute of Agrobiological Sciences, Japan, 3Research Institute for Biological Science Okayama, Japan

In the post-sequencing era, the identification of gene function is a new goal for researchers in the field of functional genomics. The identification of the function of each gene is important not only for basic research but also for applied science, especially with regard to crop research. To rapidly and efficiently elucidate the useful traits of rice, we developed a system named FOX hunting (Full-length cDNA Over-eXpressor gene hunting) that uses full-length cDNAs. Since full-length cDNAs contain all the information of functional mRNAs and proteins, we used full-length cDNAs for systematic gain-of-function mutations by introducing them into plants. We used Arabidopsis as a host for the production of rice FOX Arabidopsis mutant lines because it affords advantages such as efficient *in planta* transformation and short generation time. We generated more than 20,000 rice FOX Arabidopsis mutant lines representing 13,000 rice genes. These lines were screened systematically for various traits such as morphology, photosynthetic ability, UV resistance, element composition, production of plant hormones and secondary metabolites, fungal and bacterial resistance, and heat and salt tolerance. This database was named the 'RIKEN rice FOX Arabidopsis mutant database'. It contains around 18,000 records of rice FOX Arabidopsis mutants, and allows users to search against all the screening results. The number of searchable items is approximately 100; moreover, the database can be searched using rice and Arabidopsis gene/protein IDs, and sequence similarity searches can be performed for traits as well as for the full-length rice cDNAs that were introduced into Arabidopsis. This database is available at http://ricefox.psc.riken.jp/

P505 GABI-Kat: Keeping the standard high in an intensively used T-DNA insertion line collection

Gunnar Huep1, Nils Kleinboelting1, Andreas Klassen1, Prisca Viehoever1, Heinz Saedler2, Bernd Weisshaar1

1CeBiTec, Bielefeld University, Bielefeld, Germany, 2MPI for Plant Breeding Research, Cologne, Germany

Initiated in 1999 at the MPI for Plant Breeding Research and further developed until today at Bielefeld University, the GABI-Kat collection provides scientists around the world with T-DNA insertion mutants of *Arabidopsis thaliana* for reverse genetics. It is one of the largest publicly available insertion mutant resources worldwide and of major importance for gene function search projects in plant genomics. About 90,000 T-DNA mutagenised lines with sequence-characterised insertions sites have been generated. The resulting FSTs (flanking sequence tags) were mapped to the *A. thaliana* genome using BLAST, and the corresponding locus (gene) annotation was deduced for each insertion. The results were integrated into an FST database describing which genes have been disrupted (http://www.gabi-kat.de/).

Users of the GABI-Kat collection are provided with confirmed insertion lines, which means that the FST-based prediction of the given insertion allele is verified in the offspring generation (mostly T2) before delivering the seeds. To secure the population, to enable easy access to confirmed alleles, and to reduce the workload at GABI-Kat for maintaining lines for distribution, seeds are donated to the Nottingham Arabidopsis Stock Centre (NASC).

GABI-Kat donates sets of T3 seed of confirmed lines, which include in most cases a separate seed lot of a single T2 plant that was homozygous for the insertion. In addition to user requests more than 2,500 predicted insertion sites, which are known to be unique to the GABI-Kat collection, have been analysed up to now. Of those more than 2,000 have already been donated to NASC. Taking the confirmation rate of about 80% into account, this means that most of the available unique alleles are already at NASC. In total, almost 7,000 confirmed lines represented by 96,294 individual seed bags have been donated.

P506 Transport of auxins in Arabidopsis cell suspension

Daniela Seifertova, Jan Petrasek, Eva Zazimalova

Institute of Experimental Botany AS CR, Rozvojová 263, 165 02, Prague 6, Czech Republic

Polar transport of the plant hormone auxin is an important process determining many developmental processes in plants. Most of the knowledge on genetic and developmental aspects of polar auxin transport comes from Arabidopsis plants and/or its organs and tissues. However, at the cellular level, kinetic parameters of auxin transport across membranes were described using predominantly suspension-cultured tobacco cells. It was shown there that naphthalene-1-acetic acid (NAA) penetrates into cells by passive diffusion and it is actively transported out of cells. On the contrary, another synthetic auxin 2,4-dichlorophenoxyacetic acid (2,4-D) enters cells only actively and it is a very weak substrate for efflux carriers. Native auxin, indole-3-acetic acid (IAA), is transported, depending on its dissociation form, both passively and actively into the cell and as an anion at intracellular ph 7.0 it is transported only actively via efflux carriers out of the cell.

In Arabidopsis cell lines kinetic parameters of cellular auxin flow are completely missing. Here, we show the characteristics of auxin transport using Arabidopsis cell suspension established by May and Leaver (1993) cultured at continuous darkness and absence of cytokinins in culture medium. This cytokinin-independent and auxin-dependent cell line is therefore comparable with respect to hormonal requirements to tobacco cell suspension BY-2. Besides, the fact that this suspension is dependent on external addition of auxins is advantageous for the study because of the low interference with endogenous auxin. Our results on Arabidopsis cells indicate a higher rate of actively transported auxin molecules into and out of cells compared to tobacco cells. Moreover, not only rate of the active transport but also specificity towards particular auxins seems to be different between these two populations of cycling cells reflecting possibly species-specific differences.

May, M.J. & Leaver, C.J. Plant Physiol. 103, 621-627 (1993).

Acknowledgements: The work was supported by the Ministry of Education, Youth and Sports of the Czech Republic, project no.: LC06034.

P507 Improvement of the generation of stable knockdown lines using primary synthetic microRNAs (pri-smiRNAs) in Arabidopsis

Sandra Niemeier, Thomas Merkle

Institute of Genome Research and Systems Biology, Faculty of Biology III, Bielefeld University, D-33594 Bielefeld, Germany

In plants, microRNAs (miRNAs) typically show high sequence complementarity to their target mRNAs and act as highly specific post-transcriptional regulators of the genes or gene families they target. As a functional genomics approach, this characteristic is exploited to design and express synthetic miRNAs (smiRNAs) that specifically target single genes or members of gene families simultaneously in order to generate transgenic lines that show stable down-regulation of their expression.

We generated pri-smiRNAs on the basis of the primary transcript of the Arabidopsis *MIR159a* gene by replacing the original *miR159a* and the complementary *miR159a** with novel 21 nt-long sequences, keeping the overall secondary structure as predicted by the program *RNAfold*. For the optimization of the smiRNA design, we used the program *RNAhybrid* that allows for thorough screening in the complete Arabidopsis transcriptome for possible off-targets that might not be recognized by other means. To improve the molecular cloning of the pri-smiRNA, which is done by two consecutive overlap-extension PCR reactions, we inserted restriction sites in the original *MIR159a* primary transcript to accommodate the smiRNA/smiRNA* cDNA fragment, and experimentally proved the suitability of this approach.

As a proof of concept, we targeted the single gene encoding chalcone synthase (*CHS*) in Arabidopsis. We demonstrate smiRNA (*CHS*) expression and *CHS* mRNA cleavage in different transgenic lines. Phenotypic changes in these lines were quantified with respect to anthocyanin and flavonol content. The manifestation of chs loss-of-function phenotypes in different transgenic lines ranged from mild to severe, similar to that of a *chs* knockout mutant (*tt4*). We show that this difference is due to variations in smiRNA (*CHS*) expression levels in these lines. Using this experimental setup we also generated four additional versions of smiRNA (*CHS*) showing lower sequence complementarity to the *CHS* mRNA target site in order to test how many modifications are tolerable for smiRNA(CHS) function.

P508 Comprehensive prediction of novel microRNA targets in *Arabidopsis thaliana*

Leonardo Alves-Junior1,2, Sandra Niemeier1, Arne Hauenschild3, Marc Rehmsmeier3,4, Thomas Merkle1

1Institute of Genome Research and Systems Biology, Faculty of Biology, Bielefeld University, D-33594 Bielefeld, Germany, 2John Innes Centre, Dept. of Cell and Developmental Biology, Norwich Research Park, Colney, Norwich NR4 7UH, UK, 3Bioinformatics of Regulation, Center for Biotechnology (CeBiTec), Bielefeld University, D-33594 Bielefeld, Germany, 4GMI - Gregor Mendel Institute of Molecular Plant Biology GmbH, Dr. Bohr-Gasse 3, A-1030 Vienna, Austria

MicroRNAs (miRNAs) are 20-24 nucleotides long endogenous non-coding RNAs that act as post-transcriptional regulators in metazoa and plants. Plant miRNA

targets typically contain a single sequence motif with near-perfect complementarity to the miRNA. Here, we extended and applied the program *RNAhybrid* to identify novel miRNA targets in the complete annotated Arabidopsis transcriptome. *RNAhybrid* predicts the energetically most favorable miRNA:mRNA hybrids that are consistent with user-defined structural constraints. These were: (i) perfect base pairing of the duplex from nucleotide 8 to 12 counting from the 5´end of the miRNA; (ii) loops with a maximum length of one nucleotide in either strand; (iii) bulges with no more than one nucleotide in size; and (iv) unpaired end overhangs not longer than two nucleotides. G:U base pairs are not treated as mismatches, but contribute less favorable to the overall free energy. The resulting hybrids were filtered according to their minimum free energy, resulting in an overall prediction of more than 600 novel miRNA targets, in addition to already predicted/validated targets. The specificity and signal-to-noise ratio of the prediction was assessed with either randomized miRNAs or randomized target sequences as negative controls. As a test for our prediction parameters, ten targets were subjected to experimental validation, five of which were confirmed. Comparison of the GO molecular function classes of our predicted targets with those of previously predicted/validated targets shows that miRNA target genes are not strongly over-represented in any specific functional class such as transcription factors. Finally, we performed miRNA target predictions for 12 additional plant species.

P509 Specific labelling of cell types, structures and developmental stages in *Arabidopsis thaliana*

J Grønlund1, S Kumar1, S Dhondt2, G Beemster3, P Hilson2, J Beynon1, V Buchanan-Wollaston1

1WHRI, University of Warwick, UK, 2VIB, Ghent University, Belgium, 3Dept. Biologie, Universiteit Antwerpen, Belgium

Although our lives are dependent on plants, our understanding of how they grow and how different levels of organisation (i.e. whole plant, organ, cell, molecular module and molecule) are linked is still not understood. Therefore, the AGRON-OMICS consortium is using existing and novel tools to collect data that enable us to model the growth of the Arabidopsis leaf under non-limiting and limiting environmental conditions (e.g. drought).

After initiation of the leaf primordial, biomass accumulation is controlled mainly by cell proliferation and expansion in the leaves. However, the Arabidopsis leaf is a complex organ made up of at least 18 individual cell types (10 epidermal, 3 mesophyll and 5 vascular) and 11 structures. At the same time, the growing leaf contains cells at different stages of development with the cells furthest from the petiole being the first to stop expanding and undergo senescence. Sampling entire leaves can therefore give a distorted view of what is going on in only a subset of the cells.

Recently, sectioning and GFP lines, expressing GFP in a cell type specific manner, was used to demonstrate this effect in root tips. It was shown that the cell identity and distance from the root tip had a significant effect on the expression profiles of a large number of genes (Birnbaum *et al*, 2003; Brady *et al*, 2007). Also, lines containing a cell-type specific GAL4 trans-activation system were used to show that xylem-pole pericycle cells are necessary for lateral root development (Laplaze *et al*, 2005).

The two examples mentioned above show the power of such tools. We are therefore using the LhG4/*pOp6* transactivation system, to develop lines that will allow us to label all the specific cell types, structures and developmental stages in the Arabidopsis leaf. Combined with laser microdissection, this allows us to create a high-resolution expression map of the leaf and to specifically overexpress or repress transcription of genes in a spatial and temporal manner.

P510 AGRONOMICS1 – A new resource for Arabidopsis transcriptome profiling

Hubert Rehrauer1, Catherine Fournier1, Naira Naouar2, Andrea Patrignani1, Stephane Rombauts2, Sascha Laubinger3, Georg Zeller3, Stefan Henz3, Yves Van de Peer2, Marnik Vuylsteke2, Detlef Weigel3, Pierre Hilson2, Wilhelm Gruissem1, Lars Hennig1

1ETH Zurich, Swiss Federal Institute of Technology, Zurich, Switzerland, 2Department of Plant System Biology, VIB-Ghent University, Ghent, Belgium, 3Department of Molecular Biology, MPI-DB, Tuebingen, Germany

During recent years, transcriptome profiling has become a routine technique in biology. For transcriptome profiling in Arabidopsis, the Affymetrix ATH1 array is most commonly used. However, this microarray contains only 22810 probesets, because many probesets are ambiguous, only 21000 genes gene-specific estimates of transcript levels are provided. The TAIR8 genome annotation contains more than 33000 annotated genes; thus the ATH1 array profiles only about 65% of them.

We have developed AGRONOMICS1, a new Affymetrix Arabidopsis microarray. The AGRONOMICS1array is a tiling array, which contains the complete paths of both genome strands with on average one 25mer probe per 35bp genome sequence window. In addition, the new AGRONOMICS1array contains all perfect match (PM) probes from the original ATH1 array. The AGRONOMICS1array can be used for diverse functional genomics application such as (i) reliable expression profiling of more than 31000 genes, (ii) transcript discovery, (iii) splice variant detection and (iv) chromatin immunoprecipitation coupled to microarrays (ChIP-chip).

Here, we describe the design of the new AGRONOMICS1array and compare its performance to the ATH1 array.

We found that results from both microarrays are of similar quality, but that AGRONOMICS1arrays yield expression information for considerable more genes. Usage of the ATH1 probes on AGRONOMICS1arrays allows generating results that closely mirror ATH1 array-based results.

P511 A quantitative approach to monitor amiRNA-mediated RNA interference in Arabidopsis protoplasts

Claude Becker, Alexander Dovzhenko, Dominik Lenz1, Klaus Palme

University of Freiburg, Freiburg, Germany

Artificial microRNAs (amiRNAs) are a tool for targeted single- or multiple-gene knock-down in plants and can be designed against any gene of the Arabidopsis genome. However, efficacy of amiRNAs on a whole-plant level has proven to be variable. Arabidopsis protoplasts constitute a system in which molecular and physiological processes can be monitored and evaluated in a simplified, well-defined environment. We have developed a protoplast-based system that allows the functional characterization of amiRNAs and the assessment of knock-down levels of the target gene by a fluorescent read-out, in correlation with protein and RNA data. We have established a method for high-throughput transformation of protoplasts, computational identification of transformed cells and automated analysis of their respective fluorescence levels. By using a newly generated vector system, we have been able to down-regulate stably as well as transiently expressed transgenes by transiently expressing amiRNAs in the cells. We could identify classes of amiRNAs leading to different expression levels of the auxin efflux carrier AtPIN1, indicating variable amiRNA efficacy.

Our system provides a fast way to identify functional amiRNAs prior to their application on a whole-plant level. In combination with bioinformatical analysis of amiRNA and target site characteristics, our approach allows the identification of new parameters for amiRNA design and can give new insights into RNA interference mechanisms.

P512 The Arabidopsis Biological Resource Center – 2009 activities and stock holdings

Randy Scholl, Emma Knee, Deborah Crist, Luz Rivero, Christopher Calhoun, Natalie Case, Juan Castro, James Mann, Julie Miller, Bhuma Muthuvel, Garret Posey, Ridhima Shah, Pamela Vivian, Damitha Wickramasinghe, Hehua Yan, Zhen Zhang

The Ohio State University, Columbus, (Ohio), USA

ABRC has been collecting, preserving and distributing seed and DNA stocks of Arabidopsis since 1991. The core of our seed stock collection is 3,400 mutant lines. Recombinant inbred populations, natural accessions and other species are also distributed. Stocks relevant to functional genomics include flank-tagged insertion collections, representing more than 25,000 loci. These are comprised of 1) T-DNA lines from Salk Institute (J. Ecker lab), Syngenta Biotechnology, GABI-Kat, and U. of Wisconsin; and 2) transposon lines from Cold Spring Harbor Laboratory, Institute of Molecular Agrobiology, and John Innes Centre. RNAi lines from the Chromatin Functional Genomics and AGRIKOLA Consortia are also available.

The DNA collection includes over 31,000 cDNA entry clones, representing approximately 16,000 loci. This is comprised of donations from SSP (J. Ecker, A. Theologis, R. Davis), Salk (J. Ecker), TIGR (C. Town), Peking/Yale (X. W. Deng), J. Callis, and the Arabidopsis Membrane Interactome Project. In addition, over 11,000 expression constructs from S. P. Dinesh-Kumar, S. Clouse, and J. Doonan are available. 8,816 amiRNA clones have been received from G. Hannon, and more are expected. 241 versatile vectors for Gateway™ and other systems, and 1,027 cloned genes and constructs are also distributed.

Initiatives involving confirmed T-DNA insertion lines include distribution of nearly 30,000 SALK, SAIL, WiscDsLox, and Gabi-Kat lines verified by J. Ecker and individual researchers. A complete set of confirmed insertion lines from J. Ecker is being prepared for large phenotypic studies.

ABRC is supported by the National Science Foundation

P513 The NASC Affymetrix service

Daisy Belfield, Marcos Castelallanos, Zoe Emmerson, Graeme Gill, Neil Graham, Nick James, Lubomira Kacinova, Nicola Leftley, Bob Parkinson, Rebecca Roberts, Beatrice Schildknecht, Sean May

University of Nottingham, Loughborough, UK

The NASC Affymetrix service (http://affy.arabidopsis.info/) has been running since 2000 and has processed over 3000 arrays. This is a complete service, with users providing total RNA and includes QC tests, labelling, hybridisation, scanning etc. The data is then sent to the user on CD or via an ftp site and is also added to our in house NASCArrays database and submitted to both the GEO and ArrayExpress databases. Assistance with experimental design and data analysis can also be provided as part of the service. All Affymetrix arrays including expression, tiling, miRNA and SNP arrays can be processed. New arrays available include an Arabidopsis SNP array, Brassica, tobacco, Brachypodium and miRNA expression arrays.

All the Arabidopsis data generated from the service is made publicly available in our MIAME compliant NASCArrays database. This database now contains array results, both from the NASC service and from donated data, covering a wide range of experiments including mutant analysis, developmental series, chemical treatment and the AtGenExpress project experiments.

The experiments can be searched or browsed based on experiment type, researchers name, date performed etc. The data can be downloaded either as raw (.cel files) or MAS5 normalised data (excel files). There are a number of tools associated with the database that can be used to analyse the data, for example spot history and genes swinger. The data can also be accessed and analysed in detail using the Genespring workgroup database. This will allow users to perform detailed using the Genespring GX software from Agilent. The types of analysis include ANOVA tests, volcano plots, clustering, GO and pathway analysis.

P514 Rapid and efficient analysis of protein subcellular localization and interactions using transient transformation of Arabidopsis seedlings

Jessica Marion, Mehdi Khafif, Yannick Bellec, Lien Bach, Lionel Gissot, Jean-Denis Faure, Christian Meyer

Institut Jean-Pierre Bourgin (IJPB), INRA Centre de Versailles, Versailles, France

A comprehensive and accurate characterization of the functions of Arabidopsis genes relies on our ability to determine, and validate *in planta*, protein localization and interaction networks in a rapid and reliable way. This can be performed by transient expression of fluorescent protein (FP)-based constructs in an organized plant organ in order to address the problem of tissue-specific expression of protein complexes. FP fused to candidate proteins have been widely used to monitor protein subcellular localization and protein-protein interactions via techniques like Bimolecular Fluorescent Complementation (BiFC). Tobacco epidermal cells have already been used to express FP-based constructs using Agrobacterium infiltration, but they represent a heterologous system for the expression of Arabidopsis proteins and, like cell culture, cannot be utilized to test expression in other genetic backgrounds like mutant or marker lines. Therefore the availability of an efficient and rapid transient expression system in Arabidopsis for FP-based constructs would be essential for an extensive functional analysis of protein networks and localizations in plant tissues.

We will present an *in planta* transient transformation technique that allows single or multiple expression of constructs containing various FP tags in Arabidopsis cotyledons using vacuum infiltration of Agrobacterium into young seedlings (Marion *et al*, 2008, Plant J 56, 169–179). This method is versatile, allowing the use of pre-existing binary vectors, quick and efficient since we obtained the expected subcellular distribution of reference markers in various compartments (cytosol, nucleus, plasma membrane and golgi etc). Co-transformation with two different Agrobacterium strains was obtained at a high frequency, thus this method can be used for co-localization with subcellular markers as well as BiFC studies to establish protein interaction networks.

This work was supported by the EU FP6 AGRON-OMICS consortium.

P515 High complexity, random-primed domain libraries for yeast two-hybrid analysis of the *A. thaliana* interactome

Hélène Kiefer1, Petra Tafelmeyer1, Maryline Masson1, Emilie Vinolo1, Jean-Christophe Rain1, Nam-Hai Chua2, Heribert Hirt3, Jens D Schwenn4, Martin F Yanofsky5, Laurent Daviet1, Etienne Formstecher1

1HYBRIGENICS, Paris, France, 2Rockefeller University, New York, USA, 3URGV Plant Genomics, Evry, France, 4Ruhr University, Bochum, Germany, 5USCD, La Jolla, USA

Yeast two-hybrid (Y2H) protein interaction screening has proven instrumental in the analysis of the model plant *Arabidopsis thaliana* interactome, mostly thanks to pairwise testing[1] or screening of oligo dT-primed cDNA libraries.[2,3] However, interaction map completeness has been limited by the use of full-length proteins and C-terminal polypeptide fragments which result in significant false negative rates.

To circumvent these limitations, we have used a domain-based strategy to construct two highly complex, random-primed cDNA libraries. The first library has been prepared from one-week-old seedlings grown *in vitro* at 24°C with 16 hours of light per day. The second library was obtained by combining opened and unopened flowers. The complexity of both libraries is greater than 10 million independent fragments in yeast, with an average fragment size of 800 bp.

To ensure reproducible and exhaustive Y2H results, these libraries are screened to saturation using an optimized mating procedure. This allows to test on average 100 million interactions per screen, corresponding to a 10-fold coverage of the library. As a consequence, multiple, independent fragments are isolated for each interactant, enabling the immediate delineation of a minimal interacting domain and the computation of a confidence score.[4]

These two *A. thaliana* libraries have been integrated into our high-throughput yeast two-hybrid platform and are available for screening on a fee-for-service basis. More than 130 screens for 75 distinct research groups have been completed so far. Results from representative screens performed on both libraries will be presented at the meeting.

1 Gremski K. *et al* 2007, Development, 134:3593
2 Andreasson E. *et al* 2005, EMBO Journal, 24:2579
3 Lillig C.H. *et al* 2001, Archives of Biochemistry and Biophysics, 392:303
4 Formstecher E. *et al* 2005, Genome Research, 15:376

P516 NASC Germplasm resources and annotation

Daisy Belfield, Marcos Castellanos, Zoe Emmerson, Graeme Gill, Neil Graham, Nick James, Lubomira Kacinova, Nicola Leftley, Bob Parkinson, Rebecca Roberts, Beatrice Schildnecht, Sean May

University of Nottingham, Loughborough, UK

The European Arabidopsis Stock Centre (NASC) along with ABRC (Arabidopsis Biological Resource Center) in the US, stores and maintains over 600,000 stocks. These stocks are distributed globally. Our stocks include a large proportion of insertion mutation lines approaching saturation of the transcriptome, ecotypes, mapping lines, activation tagged inserts, and promoter/enhancer traps.

Recent and ongoing donations include the Gabi Kat lines, WAVE marker stocks, and pGreen vectors. Confirmed homozygous T-DNA insertion lines from the SALK institute and from other groups are being distributed. A number of smaller donations have also been received from a number of groups, and further donations are encouraged.

All of these seeds can be ordered through our online catalogue at http://arabidopsis.info

NASC are also keen to promote the use of ontologies and standards to enhance the annotation of the data stored in the germplasm catalogue. One of the first steps has been to use Plant Ontology (PO) with the Phenotype and Trait Ontology (PATO) to curate germplasm phenotype data.

Ontologies allow us to standardise the way we describe phenotypes observed in mutant plants. The annotations allow users to search for specific phenotypes and will also retrieve information about mutants that share a similar phenotype. This was almost impossible before ontologies were implemented, as the phenotypes are stored in a free text format making them difficult to search and compare.

We are now encouraging all donors to use the ontologies to describe any observed phenotypes.

P517 Transcriptomic responses of Arabidopsis to post-emergence herbicides targeting diverse cellular processes

Scott Baerson1, Zhiqiang Pan1, Julie Blessitt1, Ameeta Agarwal2, Stephen Duke1

1USDA-ARS Natural Products Utilization Research Unit, P.O. Box 8048, University, MS 38677 USA, 2National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677 USA

Herbicides represent approximately 70% of the synthetic pesticides currently in use, however the available chemistries target a relatively limited number of plant pathways and enzymes. Thus, there is a significant need to expand upon the existing repertoire of tools available to growers for weed management programs. Numerous products derived from secondary metabolic pathways possess biological activities that could potentially be exploited for use as natural product-based herbicides, furthermore, many of the phytotoxic compounds produced by plants and other organisms inhibit cellular processes distinct from those targeted by commercial herbicides. We have therefore initiated a program to identify natural product-based plant growth inhibitors possessing novel modes of action, and have incorporated transcriptional profiling as one of several diagnostic tools used. Transcriptional profiling has become a mainstay for clinical drug discovery efforts, where it is typically used in combination with other approaches to generate transcriptome 'fingerprints' associated with specific toxicant chemistries. We have adopted a similar approach to identify natural product-derived herbicides possessing novel modes-of-action, and to facilitate these efforts have begun compiling transcriptome 'fingerprints' for the major synthetic herbicides currently in use generated under standardized conditions.

Our progress to date includes fingerprints generated for a series of post-emergence herbicides representing diverse modes-of-action, using 10-day-old Arabidopsis seedlings exposed to I50 and I99 concentrations harvested at 6, 18, and 48 h post-treatment. An overview of these screening efforts as well as preliminary analyses of the transcriptional profiling data sets generated thus far will be pre sented.

P518 The *Arabidopsis lyrata* genome – what can we learn from the close relative of *Arabidopsis thaliana*

Ya-Long Guo1, Jan-Fang Cheng2, Tina T Hu3, Georg Haberer4, Jesse Hollister5, Pedro Pattyn6, Richard M Clark1,15, Stephan Ossowski1, Korbinian Schneeberger1, Sang-Tae Kim1, Jun Cao1, Christa Lanz1, Robert P Otillar2, Joy M Bergelson7, Justin O Borevitz7, Anne E Hall8, Charles H Langley9, Barbara Neuffer10, June B Nasrallah11, Outi Savolainen12, Stephen I Wright13, Igor V Grigoriev2, Klaus F X Mayer4, Jeremy Schmutz14, Yves Van de Peer6, Brandon S Gaut5, Magnus Nordborg3, Dan Rokshar2, Detlef Weigel1

1 Department of Molecular Biology, Max Planck Institute for Developmental Biology, Tübingen, Germany. 2 DOE-Joint Genome Institute, Walnut Creek, CA, USA. 3 Molecular and Computational Biology, University of Southern California, Los Angeles, CA, USA. 4 Munich Information Center for Protein Sequences, Institute for Bioinformatics, Gesellschaft fur Strahlenforschung Research Center for Environment and Health, Neuherberg, Germany. 5 Department of Ecology and Evolutionary Biology, University of California, Irvine, CA, USA. 6 Department of Plant Systems Biology, Flanders Interuniversity Institute for Biotechnology (VIB), Ghent University, Ghent, Belgium. 7 Department of Ecology and Evolution, University of Chicago, Chicago, IL, USA. 8 Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL, USA. 9 Section of Evolution and Ecology, University of California, Davis, CA, USA. 10 Department of Systematic Botany, Universität Osnabrück, Osnabrück, Germany. 11 Department of Plant Biology, Cornell University, Ithaca, NY, USA. 12 Department of Biology, University of Oulu, Oulu, Finland. 13 Department of Biology, York University, Toronto, Canada. 14 Stanford Human Genome Center, Stanford University, Stanford, CA, USA. 15 Department of Biology, University of Utah, Salt Lake City, Utah, USA

Arabidopsis thaliana was the first plant, and the third multicellular organism after *Caenorhabditis elegans* and *Drosophila melanogaster* to have a reference strain completely sequenced. In order to leverage the extensive genomic information available for *A. thaliana*, including an increasing amount of knowledge of within-species diversity, as well as to better understand plant genome evolution in general, we produced a reference genome sequence of *Arabidopsis lyrata,* the closest well-characterized relative in the same genus as *A. thaliana*, which diverged about 5 million years ago. The genomes was ~8-fold shotgun sequenced. Comparing the genomes of *A. thaliana* and *A. lyrata* provides an unprecedented opportunity to understand key aspects of plant genome evolution in species that share high enough sequence identity that intermediate genomic changes can be directly determined. In detail, besides the comparison of the general issues, including large-scale chromosome, gene content, orthologs, gene family, repeat, we paid more attention to four aspects: elucidation of genome size variation in the two closely related plant species for the first time;

genome-wide analysis of microstructural evolution; the relationship between breeding system and genome size evolvement; SNP density comparison among different chromosomes and different populations within *A. lyrata.* We will report on our analyses, focusing on genome size variation between the two species in various aspects.

P519 AtEnsembl

Daisy Belfield, Marcos Castellanos, Zoe Emmerson, Graeme Gill, Neil Graham, Nick James, Lubomira Kacinova, Nicola Leftley, Bob Parkinson, Rebecca Roberts, Beatrice Schildnecht, Sean May

University of Nottingham, Loughborough, UK

The Arabidopsis Ensembl (AtEnsembl) genome browser is the latest iteration of a series of continuous databases that we have developed at NASC as part of UKcrop.net from 1996 to date. These 'active catalogues' have been used to capture and curate public genomic, mapping, and germplasm data describing Arabidopsis and integrate them into a form that makes our data and physical seed services more accessible and useful to the Arabidopsis community.

During the most recent funding period for this continuous database we have included a gateway to the extensive transcriptomic array data available at NASC (circa 4,000 affymetrix chips) and have recently addressed the integration of re-sequencing SNP data from varieties (>17 ecotypes to date).

In keeping with our ethos of completely open data access we have also made all data available to the general scientific community (including collaborators and competitors) via distributed (user driven) facilities and tools such as SOAPlab webservices. These allow both occasional and bulk users to have access driven by their actual needs without having to make special individual arrangements for unusual data access.

AtEnsembl now also includes distinct lanes for Brassica and Solanum (e.g. Tomato, Pepper) species in the genome browse facility to facilitate cross species access to translational resources within Arabidopsis (e.g. mutants, array data).

Plant Growth Regulators

P520 A subunit of the Anaphase-Promoting Complex (APC) affects auxin-regulated development in Arabidopsis

Donna Lindsay1, Peta Bonham-Smith2, Gordon Gray3, Troy Harkness1

1Department of Anatomy and Cell Biology, University of Sasktachewan, Saskatoon, SK, Canada, 2Department of Biology, University of Sasktachewan, Saskatoon, SK, Canada, 3Department of Plant Sciences, University of Sasktachewan, Saskatoon, SK, Canada

Initially identified for its role in regulating mitotic progression, the evolutionarily conserved Anaphase-Promoting Complex (APC) E3 ubiquitin ligase serves to target specific proteins for degradation and is associated with multiple pathways affecting cell biology. Composed of eight to 13 highly conserved proteins in all eukaryotes studied to date, less has been determined about the role of the APC, and individual subunits, in plants. Phenotypic and molecular analysis of the first APC subunit mutant described for plants, *hobbit/apc3/cdc27* (*hbt*), suggested a correlation between the APC and auxin regulation of plant development. However, a direct role was equivocal as *APC3* is encoded by two loci in Arabidopsis. The first APC subunit to be examined crystallographically, *APC10* is believed to play a direct role in targeting substrates for ubiquitination processes.

The present study indicates *APC10*, and potentially the APC complex, serves in hormone regulation of plant development, as increased expression of *APC10* in Arabidopsis induces pleiotropic phenotypes resembling auxin-affected responses, including altered embryo, seedling and flower morphology, altered transcript levels of auxin response genes and reduced levels of an auxin-associated reporter system.

We discuss potential mechanisms involved in the role of the APC in auxin regulation of plant development.

P521 Arabidopsis ATP-binding cassette transporters promote efflux of the auxin precursor indole-3-butyric acid

Lucia Strader, Bonnie Bartel

Rice University, Houston, Texas, USA

Plants have developed numerous mechanisms to store hormones in inactive but readily available states, enabling rapid responses to environmental changes.

The phytohormone auxin has a number of storage precursors, including indole-3-butyric acid (IBA), which is apparently shortened to active indole-3-acetic acid (IAA) in peroxisomes by a process similar to fatty acid β oxidation. Whereas metabolism of auxin precursors is beginning to be understood, the biological significance of the various precursors is virtually unknown.

We found that mutation of *PDR8/PEN3/ABCG36*, encoding a plasma membrane localized ATP-binding cassette transporter, specifically restores IBA, but not IAA, responsiveness to auxin signaling mutants. Moreover, both *pdr8* and *pdr9/abcg37* mutants confer hypersensitivity to a subset of auxins. Further, we found that *pdr8* and *pdr9* mutants display defects in efflux of the auxin precursor IBA. *pdr8* also displays developmental defects in root hair and cotyledon expansion that reveal previously unknown roles for IBA-derived IAA in plant growth and development.

Our results imply that limiting IBA accumulation via PDR8- or PDR9-promoted efflux contributes to auxin homeostasis.

P522 Effects of gibberellin on the growth and gene expression in the Arabidopsis root

Haniyeh Bidadi1 Masashi Asahina2, Shinjiro Yamaguchi3, Shinobu Satoh4

11Graduate School of Life and Environmental Sciences, University of Tsukuba, Ibaraki 305-8572, Japan, 2 Department of Biosciences, Teikyo Univeristy 1-1Toyosatodai, Utsunomiya, 320-8551, Japan, 3 1-7-22 Suehiro, Tsurumi, Yokohama, Kanagawa 230-0045 RIKEN Yokohama Institute, Plant Science Center, 4 University of Tsukuba, Graduate School of Life and Environmental Sciences, Division of Integrative Environmental Sciences,Tsukuba, Ibaraki 305-8572, Japan

Our previous research showed that the gene expression of xylem sap lectin : XSP30 in root stele was circadian clock-controlled and the amplitude was up-regulated by the leaf-produced gibberellin in cucumber plant. In this research, a GA-deficient mutant of Arabidopsis (*ga3ox1/ga3ox2*) was used to observe the effects of gibberellin in root area. Applying GA4 on shoot area of the GA-deficient mutant showed obvious promotion in seminal root elongation and also root branching. In order to find out if gene expression in root area is affected by GA4 application, microarray analysis was performed on root samples of GA-treated or non-treated GA-deficient mutant. The results lead us to select several up and down-regulated genes, which may be expressed in root and controlled by the shoot. The expressions of the selected genes were confirmed by real-time PCR. Some of these genes may be responsible for shoot-root interactions.

P523 Cytosolic activity of SPINDLY implies the existence of a DELLA-independent gibberellin-response pathway to suppress cytokinin responses

Inbar Maymon, Yaarit Greenboim-Wainberg, Sivan Sagiv, David Weiss

Institute of Plant Sciences and Genetics, Faculty of Agriculture, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel

Specific plant developmental processes are modulated by cross talk between gibberellin (GA)- and cytokinin-response pathways. Coordination of the two pathways involves the O-GlcNAc transferase SPINDLY (SPY) that suppresses GA signaling and promotes cytokinin responses in Arabidopsis. Although SPY is a nucleocytoplasmic protein, its site of action and targets are unknown. Using chimeric GFP-SPY fused to a nuclear-export signal or to a glucocorticoid receptor, we show that cytosolic SPY promotes cytokinin responses and suppresses GA signaling. In contrast, nuclear-localized GFP-SPY failed to complement the *spy* mutation. To examine whether modulation of cytokinin activity by GA and SPY is mediated by the nuclear DELLA proteins, cytokinin responses were studied in double and quadruple *della* mutants. Unlike *spy*, the *della* mutants were cytokinin-sensitive. Moreover, when GA was applied to a cytokinin-treated quadruple *della* mutant, it was able to suppress various cytokinin responses. These results suggest that cytosolic SPY and GA regulate cytokinin responses via a DELLA-independent pathway(s). To study the mechanism by which *spy* suppress cytokinin responses in the cytosol, we tested its effect on localization of the early cytokinin-signaling components, Arabidopsis His-phosphotransfer proteins (AHPs) 1 and 2. In wild-type mesophyll protoplasts, AHPs accumulated mainly in the cytosol, while in *spy*, mainly in the nucleus. We propose that SPY is required for normal AHP cytosolic localization, which is a prerequisite for their interaction with cytokinin receptors.

P524 ABA regulation of nodulation in *Medicago truncatula*

Yiliang Ding1, Jeanne Harris2, Giles Oldroyd1

1John Innes Centre, Norwich, UK, 2Department of Plant Biology, University of Vermont, Vermont, USA

Nodulation is tightly regulated in legumes to ensure appropriate levels of nitrogen fixation without excessive depletion of carbon reserves. This balance is maintained by intimately linking nodulation and its regulation with plant hormones. It has previously been shown that ethylene and jasmonic acid are able to regulate nodulation and Nod factor signal transduction. Here we characterize the nature of abscisic acid (ABA) regulation of nodulation and its coordination with cytokinin signalling that is an essential component of nodule organogenesis. ABA acts in a similar manner as JA and ethylene, regulating Nod factor signaling and impacting on the nature of Nod factor induced calcium spiking. We show that genetic inhibition of ABA signaling through the use of a dominant-negative allele of *ABI1* leads to a hypernodulation phenotype. ABA can suppress Nod factor signal transduction in the epidermis and can regulate nodulation-associated cytokinin signaling in the cortex (Ding *et al*, 2008). Rhizobial inoculation leads to a localised accumulation of ABA as evidenced by the analysis of *RD22* induction, a marker for ABA perception. The enhanced levels of ABA corresponds both spatially and temporally with rhizobial induction of cytokinin signalling. We propose that the cross talk between Nod factor, ABA and cytokinin is a dynamic process and the interplay between these positive and negative hormonal regulators of nodulation provides a mode for the regulation of nodule formation.

P525 Nitric oxide biosynthesis and genome-wide analysis of its regulatory functions in *Arabidopsis thaliana*

Jorge Lozano-Juste, Laura Yeves, Jose Leon

Instituto de Biologia Molecular y Celular de Plantas (CSIC-UPV), Valencia, Spain

The biosynthesis of nitric oxide (NO) has been analyzed in Arabidopsis by studying the function of three putative enzyme-based pathways: nitrate reductase (NR/NIA)-, NO associated 1 (NOA1)-, and xanthine oxidoreductase (XOR)-dependent pathways. NR/NIA- and NOA1-dependent processes seem to be major contributors to the biosynthesis of NO in Arabidopsis. We have generated a triple *nia1nia2noa1-2* mutant severely impaired in NO production. Transcriptome analyses of *nia1nia2*, *noa1-2* and *nia1nia2noa1-2* mutant versus wild type plants uncovered a number of development- and defense-related processes that are potentially regulated by endogenous NO levels in Arabidopsis. Gene Ontology analysis of

differentially expressed genes in the *nia1nia2noa1-2* plants point to ABA- and salicylic acid (SA)-related responses as overrepresented in upregulated genes, whereas gibberellins-, auxin- and light-related responses were overrepresented among downregulated genes. Accordingly, we confirmed that NO regulated different ABA-modulated processes. NO-deficient mutants were hypersensitive to ABA and displayed decreased germination potential but increased dormancy and resistant to dehydration. In addition, a role for NO regulating de-etiolation was also uncovered. *nia1nia2noa1-2* plants had longer hypocotyls than *nia1nia2*, *noa1-2* and wild type plants under white or red light. PhyB- or DELLA-related mechanisms are being analyzed in the different NO-deficient mutant plants including the GA-dependent phenotype of hypocotyls under darkness.

A large-scale screening of T-DNA lines, carrying insertions in a large representation of loci of the Arabidopsis genome, is being carried out to identify genes involved in perception/signaling of NO. We have identified several T-DNA lines with partial NO insensitivity. The corresponding mutated genes are being functionally analyzed to identify new components on NO-related signaling pathways in Arabidopsis.

P526 Far-red light inhibits germination through DELLA-dependent stimulation of ABA synthesis and ABI3 activity

Urszula Piskurewicz, Eric Lacombe, Luis Lopez-Molina

University of Geneva, 30, Quai Ernest, Ansermet, Geneva 1211 Switzerland

Canopy light (light enriched in far-red) represses the early seed germination processes of testa and endosperm rupture by inactivating phytochrome photoreceptors. This elicits opposite changes in the levels of the phytohormones gibberellins (GA) and abscisic acid (ABA). GA promotes germination by enhancing the proteasome-mediated destruction of DELLA repressors. ABA prevents germination by stimulating the expression of ABI repressors. How phytochromes elicit changes in hormone levels and how GA- and ABA-dependent signals are coordinated to repress germination remains poorly understood.

We show that far-red light-dependent repression of testa rupture involves stabilized DELLA factors GAI, RGA and RGL2. In parallel, DELLA factors stimulate endogenous ABA synthesis, which blocks endosperm rupture through the transcription factor ABI3. The role of PIL5, a bHLH transcription factor stimulating *GAI* and *RGA* expression, is significant only if GA synthesis is not prevented: otherwise high GAI and RGA protein levels persist to block germination. Consistent with recent findings with RGL2, both GAI and RGA can repress testa rupture and promote ABA synthesis under white light when placed under the control of the *RGL2* promoter.

P527 A J-domain containing protein, JRF1, regulates the floral transition in Arabidopsis

Lisha Shen, Hao Yu

National University of Singapore and Temasek Life Sciences Laboratory, Singapore

The timing of the switch from vegetative to reproductive development in Arabidopsis is controlled by an intricate network of pathways, including the photoperiod, vernalization, autonomous and gibberellin (GA) pathways. The four genetic pathways converge on the transcriptional regulation of several floral pathway integrators including FLOWERING LOCUS T (FT), SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), and LEAFY (LFY). *SHORT VEGETATIVE PHASE (SVP)* has been shown to form a protein complex with FLOWERING LOCUS C (FLC) to tightly control the transcription levels of *FT* and *SOC1*. In order to further elucidate the regulatory network involving SVP, we performed yeast two-hybrid screening to identify its protein partners. J-DOMAIN REGULATING FLORAL TRANSITION1, (JRF1), was shown to interact with SVP *in vitro*. *JRF1* encodes a DnaJ-like protein, and was ubiquitous expressed in root, leaf and flowers. Loss-of-function of *JRF1* showed late-flowering phenotype. Further analysis showed that *JRF1* expression was affected by vernalization and GA treatment. GFP-JRF1 was localized to the plasma membrane and nucleus. The role of JRF1 in controlling of flowering time and its interaction with SVP are being further studied.

P528 The bHLH transcription factor SPT and DELLA proteins act together to regulate cell size of Arabidopsis cotyledons in a GA-dependant manner

Eve-Marie Josse1, Yinbo Gan2, Kelly Stewart1, Alison Gilday2, Ian Graham2, Karen Halliday1

1University of Edinburgh, Scotland, UK, 2University of York, UK

Most transcription factors from the basic-helix-loop-helix family XV have been characterised as key component in phytochrome signalling in plants.[1] Amongst them, PIF1, PIF3, PIF4, PIF5, PIF6 and PIF7 have been shown to directly interact with the phytochrome molecule.[2] PIFs also provide links to the hormones pathway. Indeed, it was recently shown that both PIF3 and PIF4 interact with GA-dependant growth repressor DELLA proteins to modulate hypocotyl elongation.[3,4]

We have previously shown that SPATULA (SPT), a non phytochrome-binding PIF3 homologue, is a light-stable repressor of germination, that participates in integrating light and temperature signals to control seed dormancy, through the regulation of GA production.[5] Additionally, we showed that SPT action is not restricted to germination but, like PIF3 and PIF4, that it also controls seedling development.

Here, we describe the mechanism of action of SPT in the regulation of cotyledon expansion during seedling de-etiolation. As well as examining SPT targets, we will show that SPT acts in an integrative manner, together with other

protein partners, to control cell expansion. A dual function for SPT, both as a transcription factor and as a protein stability regulator, will be discussed.

1 G. Toledo-Ortiz, E. Huq, P. H. Quail, Plant Cell 15, 1749 (2003).
2 G. Bae, G. Choi, Annu Rev Plant Biol 59, 281 (2008).
3 S. Feng *et al*, Nature 451, 475 (2008).
4 M. de Lucas *et al*, Nature 451, 480 (2008).
5 S. Penfield *et al*, Current Biology 15, 1998 (2005).

P529 Local action of GOLVEN secretory peptides stabilizes an auxin efflux carrier during plant gravitropic responses

Ryan Whitford, Ana Fernandez, Amparo Cuellar, Jurgen Kleine-Vehn, Ruth De Groodt, Steffen Vanneste, Tom Beeckman, Jiri Friml, Pierre Hilson

VIB-Ugent, Gent, Belgium

We have identified a novel plant specific peptide family called *GOLVEN (GLV). GLV* members are expressed in shoot tissues as well as the root apex. They carry an N-terminal signal peptide and a C-terminal conserved motif, dubbed the *GLV* motif. Application of synthetic peptides carrying this conserved motif (GLVp) as well as gain- or loss-of-function *glv* mutants display hypocotyl and root agravitropic phenotypes. Interestingly, *GLV1* and *GLV2* are transcribed asymmetrically in bending gravistimulated hypocotyls. Furthermore, GLV peptide application disturbs the asymmetric auxin distribution in gravistimulated organs. We will show data indicating that GLV interference with plant gravitropic processes is explained by the stabilization of auxin efflux carriers in the plasma membrane through the inhibition of vacuolar targeting without affecting cellular polarity.

Our observations reveal a novel mechanism by which a phytohormone signal is modulated through the local action of secretory peptides and suggests that phytohormones and peptide hormones may form regulatory feedback loops that reinforce developmental patterns.

P530 A transcriptional cascade mediates the Arabidopsis cytokinin response

Kristine Hill1, Dennis E Mathews2, G Eric Schaller1

1Dartmouth College, Hanover, NH, US, 2University of New Hampshire, Durham, NH, USA

Cytokinins play essential roles in plant morphogenesis. Elevated levels of cytokinin release apical dominance, inhibit root elongation, delay senescence, and enhance shoot regeneration in cultured tissues. The cytokinin response is mediated by a two-component signaling pathway that culminates in regulation of the type-B response regulators (type-B ARR family). The type-B ARRs are transcription factors that contain a receiver domain with a conserved aspartate residue that allows for regulation through phosphorylation. Mutational analysis indicates that the type-B ARRs are the primary transcription factors regulating the plant's response to cytokinin. We are performing a functional analysis to elucidate the features of the type-B ARRs that are essential to this regulation, in particular we have expressed different type-B ARRs under the control of the *ARR1* promoter to determine which can rescue an *arr1,12* mutant phenotype. Among the primary response genes regulated by the type-B ARR's are many families of transcription factors, suggesting that the type-B ARRs operate at the top of a transcriptional cascade. These families of transcription factors are frequently differentially expressed in the root and shoot, indicating that transcriptional cascades are tailored to tissue specific responses. We have taken a mutant-based approach to elucidate the subset of roles played by these primary response transcription factors in cytokinin signaling.

P531 High-throughput functional genomics of cell wall dynamics during root growth and development

Tara Holman1, Michael Wilson1, Darren Wells1, Iben Sorrensen2, William Willats2, Paul Knox3, Michael Holdsworth1, Malcolm Bennett1

1CPIB, University of Nottingham, UK LE12 5RD, 2University of Copenhagen, Denmark, 3University of Leeds, UK LS2 9JT

The Centre for Plant Integrative Biology (CPIB) aims to create a dynamic, multi-scale, model-based 'virtual' root, integrating hormone-related genetic signalling networks with changes in cell/tissue mechanical properties. These factors ultimately lead to altered growth properties of the organ.

We have divided the root into five distinct developmental sections – the meristem; the accelerating elongation zone; the decelerating elongation zone; a reference zone of mature tissue; and the rest of the root where lateral root primordia are forming. Cells in each of these five zones are undergoing discrete developmental processes – division (meristem), expansion (elongation zones) or differentiation (lateral root primordia zone).

In order to link gene expression to dynamic changes in the mechanical properties of the cell wall, we have produced genome-scale transcriptomic datasets to identify gene-expression fingerprints for each developmental section. In addition, we have used an antibody-based approach to identify changes in the cell wall glycome. The components of the cell wall are, in part, a result of the activity of cell-wall modification enzymes (e.g. pectin methyl esterase and xyloglucan endotransglycosylase/hydrolase enzymes). As the cell wall is modified, the mechanical properties are altered, which potentiates cell expansion and drives organ growth.

We are using a functional genomics approach to couple our gene-expression and cell wall glycome datasets. We aim to link these to hormone signalling data that have also been produced, and to connect hormone regulated gene signalling networks with changes in the mechanical properties of the cell wall in different developmental regions of the root.

P532 Regulation of cell morphology by auxin

Angharad Jones1, Eric Kramer2,3, Kirsten Knox4, Ranjan Swarup5, Colin Lazarus1, Ottoline Leyser4, Claire Grierson1, Malcolm Bennett3,5

1School of Biological Sciences, University of Bristol, Bristol, UK. 2Bard College at Simon's Rock, Massachusetts, USA., 3Centre for Integrative Plant Biology, University of Nottingham, Nottingham, UK., 4Department of Biology, University of York, York, UK., 5School of Biosciences, University of Nottingham, Nottingham, UK

Multicellular organisms consist of many distinct cell types. A central question in biology is how the differentiation of specialised cell types is regulated. In plants the hormone auxin appears to be the main architect of this process. Different concentrations of auxin switch on different developmental processes, and there is evidence that gradients of auxin spatially restrict developmental processes at both the tissue[1] and the cellular level.[2] Auxin gradients are created by active auxin transport, which regulates the amount of auxin entering and leaving individual cells. As the plant develops so does the distribution of auxin transporters and the pattern of auxin flow.[3] The development of root-hair cells in the epidermis of the root provides an opportunity to study how auxin regulates differentiation. Based on the distribution of auxin transporters we have produced a predictive map of auxin distribution in the root epidermis.[4]. Here experimental data will be presented showing how differentiation of auxin signalling affects root-hair development. In particular, results show that non-hair cells supply auxin to differentiating root-hair cells and contribute to the regulation of root-hair length[4] and that differences in auxin signalling in hair and non-hair cells determines their ability to produce a polar response.

1 Grieneisen, V.A., *et al* (2007) Nature 449, 1008-1013.
2 Fischer, U., *et al* (2006) Curr Biol 16, 2143-2149.
3 Xu, J., *et al* (2006) Science 311, 385-388.
4 Jones, A.R., *et al* (2009) 11, 78-84.

P533 Gibberellin signaling controls cell proliferation rate in Arabidopsis

Patrick Achard1, Andi Gusti1, Soizic Cheminant1, Malek Alioua1, Stijn Dhondt2,3, Frederik Coppens2,3, Gerrit Beemster2,4, Pascal Genschik1

1IBMP-CNRS, Strasbourg, France, 2Plant System Biology, Gent, Belgium, 3Dept of Molecular Genetics, Gent University, Belgium, 4Dpt of Biology, Antwerp University, Belgium

Plant organs are defined in apical meristems and then grow by coordinated cell division and cell expansion. This coordination involves both intra- and inter-cellular signals (hormones and other factors) and exogenous factors such as moisture, ambient temperature and light. One of the most potent endogenous regulators of plant growth is a class of hormones known as the gibberellins (GA). It is established that GA promote growth through cell expansion by stimulating the destruction of the nuclear growth-repressing DELLA proteins (DELLAs). Thus mutants accumulating DELLAs are dwarf, a phenotype that is reverted by the lack of DELLA function. However, whilst DELLAs undoubtedly restrain growth via their effects on cell expansion, their effects on cell proliferation remain unknown. Kinematic analysis of leaf and root meristem growth revealed a novel function of DELLAs in restraining cell production. Moreover, by visualizing cell-cycle markers in GA-signaling mutants, we show that GA control cell cycle activity in the root meristem via a DELLA-dependent mechanism. Accordingly, expressing *gai* (a non-degradable DELLA protein) solely in root apical meristem reduced substantially the rate of meristematic cell division and in consequence the growth of the root. We also show genetically that DELLAs restrain cell proliferation by enhancing the levels of cell cycle inhibitors. Therefore, DELLAs exert a general plant growth inhibitory activity by restraining both cell proliferation and expansion rates, thereby enabling phenotypic plasticity.

P534 Modelling auxin response

Martin Kieffer1, Gary Mirams2, Sarah Lee 3, Lynne Armitage1, Alistair Middleton2, Sara Peterson4, Karin Ljung4, Richard Napier3, John King2, Stefan Kepinski1

1Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK, 2Division of Theoretical Mechanics and CPIB, University of Nottingham, Nottingham, NG7 2RD, UK, 3Warwick HRI, University of Warwick, Warwick, CV35 9EF, UK 4 UPSC, Umeå Universitet, 90183 Umeå, Sweden

Root development is dominated by a specific distribution of auxin that correlates with proximo-distal and radial patterns of auxin-regulated phenomena from the positioning of the stem cell niche in the root apex to the transitions from cell division to elongation and then differentiation in the older root. While the phenomenology of auxin movement and distribution in the growing root and throughout much of the plant has been established by both experimental and computational methods, the link between differences in auxin concentration and outputs in various developmental contexts is not well understood. We are addressing these questions by building cell-type-specific quantitative models of the AFB-Aux/IAA-ARF system, a network of physically interacting auxin receptors and transcription factors that forms the mechanistic link between the arrival of auxin in a cell and outputs in terms of gene expression control. Here we will describe quantitative genomic, biochemical and metabolomic data used to parameterise and test models of auxin response initially in defined zones and cell-types of the root epidermis. In addition, simple preliminary models of auxin signalling have also highlighted interesting features of the system's capacity to respond to the auxin gradients and maxima.

Please note this abstract is associated with the Quantitative modelling of signalling systems on 30th June.

P535 SERKs family of co-receptors functions in multiple pathways

Catherine Albrecht1, Eugenia Russinova2, Birgit Kemmerling3, Wilma van Esse1, Rumyana Karlova1, Na Li1, Sacco de Vries1

1Wageningen University, Wageningen, The Netherlands, 2Ghent University, Ghent, Belgium, 3Tuebingen University, Tuebingen, Germany

Related receptor like kinases often have redundant functions in the same signaling pathway. Using multiple

mutant allele combinations we show that amongst the 5 members of the Arabidopsis SERK family only partial functional redundancy exists. One member of this family, SERK3, is also known as BAK1, the co-receptor of the brassinolide perceiving receptor BRI1. Only *serk* mutant alleles enhance the BR insensitivity of *serk3-1* mutant roots and hypocotyls. Independently from BRI1, only SERK1 and SERK2 proteins are essential for male microsporogenesis. SERK3 alone controls innate immunity, and together with SERK4 can also mediate cell death control in a BR-independent manner. SERK5 does not appear to have any function under the conditions tested. This shows that individual SERK proteins serve in different and independent signaling pathways.

P536 The role of salicylic acid in Arabidopsis root growth

Mary Wildermuth, Alexander Jones
University of California, Berkeley, (CA), USA

Salicylic acid (SA, 2-hydroxybenzoic acid) is a plant hormone that is known to regulate biotic and abiotic stress responses, but SA biology in the root is largely unexplored. Results from SA marker studies and known SA signaling mutants indicate that SA may have a role in root development. Other major hormones that regulate root growth and development inhibit roots when exogenously applied in the µM range. We found root growth is inhibited at 10-100µM SA, concentrations that may be of physiological relevance in endogenous stress signaling and responses to rhizosphere SA. This SA root growth inhibition results primarily from a dramatic reduction in cell elongation and is specific to SA. Structurally similar molecules such as 4-hydroxybenzoate do not inhibit root growth at these concentrations. Prominent theories of root cell elongation point to reactive oxygen species (ROS) accumulation and auxin signaling as key components of the process. Through the use of ROS specific dyes and auxin reporters, we show that root inhibitory SA eliminates certain ROS maxima and alters auxin signaling in growing root tips. These effects could explain SA impact on roots. We further show through analysis of SA signaling mutants that this inhibition does not require the well-established NPR1 SA immune response pathway. Rather, the NPR1 pathway seems to be involved in stimulating root growth.

Our data also supports a role for WRKY38, a highly SA responsive transcription factor known to be expressed in the root and modulate the NPR1 pathway, in regulating the root stimulation component of SA response. In conclusion, we have shown that known SA signaling pathways contribute to root elongation, and may be functional in normal root development. We have also shown that exogenous SA inhibits root cell elongation, abolishes certain root ROS maxima and alters root auxin signaling. Work in progress is aimed at elucidating the components of this novel SA response.

Funded by NSF Arabidopsis 2010 grant MCB-0420267 to M.C.W. and EPA STAR Pre-doctoral fellowship to A.M.J.

P537 ROP localisation by auxin in Arabidopsis root hair cells

Robert Payne, Claire Grierson
University of Bristol, Bristol, UK

Local activation of Rho GTPases is important for many functions including cell polarity, morphology, and growth. Although a number of molecules affecting Rho-of-Plants small GTPase (ROP) signalling are known, it is unclear how ROP activity becomes spatially organised. Arabidopsis root hair cells produce patches of ROP at consistent and predictable subcellular locations, where root hair growth subsequently occurs.

We present a mathematical model to show how interaction of the plant hormone auxin with ROPs could spontaneously lead to localised patches of active ROP. Our results suggest cellular and molecular features that affect correct positioning of the ROP patch, and provide a unique explanation linking the molecular biology to the root hair phenotypes of multiple mutants and transgenic lines.

P538 Calmodulin binding transcription activator (CATMA1) serves as a link between growth signals and stress responses

Yael Galon-Wolfenson1, Roni Aloni2, Dikla Nachmias2, Orly Snir2, Ester Feldmeser3, Sarah Scrase-Field4, Joy M Boyce4, Nicolas Bouche5, Mark R Knight6, Hillel Fromm2
1Tel-Aviv University, 2Tel Aviv University, 3Weizmann Institute of Science, 4Oxford university, 5INRA, 6Durham University, Department of Plant Sciences, Faculty of Life Sciences, Tel Aviv University

Ca^{2+} concentrations are transiently elevated as a response to various environmental and developmental signals. These Ca^{2+} elevations lead to various plant responses, using proteins which bind Ca^{2+} (e.g. calmodulin). One of these responses is changes in transcription regulation of genes. In Arabidopsis there are 6 CAMTA genes (*AtCAMTA1-AtCAMTA6*), which encode calmodulin-binding transcription activators.[1] Recently a few of their physiological roles in biotic and abiotic stress responses were revealed,[2,3] and also their target DNA element.[4] Here, we report that expression of Arabidopsis *CAMTA1*, is regulated by auxin and different stress signals. The responsiveness of *CAMTA1* to auxin was further confirmed by inhibition of polar auxin transport. Furthermore, the expression pattern of *CAMTA1* changed significantly and differentially upon exposure to increasing salt concentrations, heat shock, and low pH. Transcriptome analysis of a *camta1* T-DNA insertion mutant revealed 63 up- and 66 down-regulated genes, several of which are associated with auxin signaling and related physiological responses (e.g. R/FR light responses, flavonoid biosynthesis, and sulfur assimilation), and stress responses. Furthermore, analysis of etiolated hypocotyl elongation in the presence and absence of auxin revealed that *camta1* mutants are

hyper-responsive to auxin compared to wild type plants. Thus, CAMTA1 may participate in environmental stress responses and in regulating plant growth through auxin signaling.

1 Bouché N. *et al* (2002) J Biol Chem 277: 21851-21861
2 Galon Y. *et al* (2008) FEBS Letters 582: 943–948
3 Galon Y. *et al* (submitted).
4 Finkler A. *et al* (2007) FEBS Letters 581: 3893-3898

P539 Nucleus-focused proteomic analysis of Arabidopsis suspension cells treated by Brassinolide and/or Brz

Tomoaki Matsuo1, Tomoaki Shigeta1, Daisuke Yasuda1, Yuya Yoshimitsu1, Takafumi Mori1, Yasushi Nakamura2, Shigehisa Okamoto1, Tadao Asami 3, Shigeru Yoshida 4

1Department of Biochemical Science & Technology, Kagoshima University, Kagoshima, Japan, 2Department of Food Sciences & Nutritional Health, Kyoto Prefectural University, Kyoto, Japan, 3Department of Applied Biological Chemistry, University of Tokyo, Tokyo, Japan, 4Institute of Physical and Chemical Research (RIKEN), Wako, Japan

Brassinosteroids (BRs), the polyhydroxylated steroid hormones of plants, regulate the growth and differentiation of plants throughout their life cycle. They also improve some stress-tolerances for cold, heat, drought, and nutrient deficiency in various plants.

BRs are perceived at the plasma membrane by direct binding to the extracellular domain of the BRI1 receptor S/T kinase. BR perception iniciates a signaling cascade, acting through a GSK3 kinase, BIN2, and the BSU1 phosphatase, which in turn modulates the phosphorylation state and stability of the nuclear transcription factors BES1 and BZR1.

In this study, we first confirmed exogenously applied BL to promote cell growth of Arabidopsis cultured cells during 8 days of treatment and followed to analyze BR-regulated proteins in the partially purified nuclear fractions extracted specifically by Sigma Cellytic PN kit using 2-DE with two special fluorescence dyes and identified BR-regulated (increased or decreased) proteins by MALDI-TOF MS or LC/MS/MS analysis after enzymatic digestion. We also examined phosphorylation changes of the protein components in the same fractions using Pro-Q Diamond phosphoprotein staining.

After 2-D PAGE analyses, more than 551 proteins in the nuclear fractions were detected by SYPRO Ruby dye-staining and image analysis over a pH range of 4 – 9 and a size range of 10 – 100 kDa. As judged by the match mode of the PDQest software, 53 proteins were upregulated and 79 were downregulated in response to BRs levels; BL-treated vs non-treated cells and non-treated vs Brz-treated cells.

We have identified a number of BR-responsive proteins and recognized functional roles for some of the BR-regulated proteins in BR-promotion of plant growth.

P540 A dwarf mutant of *Arabidopsis thaliana* impaired in carbohydrate and gibberellin metabolism

Eleonora Paparelli1, Silvia Gonzali1, Giacomo Novi2, Sandro Parlanti2, Elena Loreti3, Lorenzo Mariotti2, Elena Degl'Innocenti4, Lucia Guidi4, Pierdomenico Perata1

1PlantLab, Scuola Superiore Sant'Anna, Pisa (Italy), 2Dipartimento Biologia delle Piante Agrarie, UniPI, Pisa (Italy), 3IBBA-CNR, Pisa (Italy), 4Dipartimento di Chimica e Biotecnologie Agrarie, UniPI, Pisa (Italy)

A dwarf mutant of *Arabidopsis thaliana* (L.) Heynh, was isolated by screening T-DNA tagged plants germinated on turanose-containing medium. The dwarf phenotype was accompanied by an altered leaf morphology and a delay in the flowering time. In the mutant, which has been called *nana* (Italian for dwarf), a T-DNA insertion was identified in the promoter region of *At3g12700*, coding for a putative aspartyl protease similar to a DNA binding protein of chloroplast nucleoids (CND41). Because of this mutation, *nana* lacks important promoter elements of the gene, such as GARE, SURE and ABRE. When grown in a 12-h photoperiod, *nana* shows alterations in *At3g12700* gene expression profile and lacks the daily fluctuation of soluble sugars levels. Plant height and leaf shape can be partially restored by application of gibberellins, indicating that a reduction in GAs content might be a major cause of dwarf phenotype. Actually, *nana* shows reduced endogenous level of ent-kaurene, a GA-biosynthesis precursor, and large diurnal changes in expression of ent-kaurene synthase compared to wild type plants. Also a moderate increase in light irradiance can induce *nana* plants to enhance their size. Therefore the rate of photosynthesis was analysed in the mutant compared to wild type, showing no detectable changes in electronic transport but a significantly higher carbamylation ratio of Rubisco. On the basis of our results, we hypothesize a role for NANA in the chloroplast as a major player in the turnover of Rubisco, whose lower levels can deeply influence sugar concentrations and, as a consequence, negatively affect gibberellin biosynthesis at the level of *ent-kaurene synthase.*

P541 ASK3, a novel regulator of brassinosteroid signalling

Juliane Mayerhofer1, Wilfried Rozhon1, Elena Petutschnig1, Shozo Fujioka2, Claudia Jonak1

1Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria, 2RIKEN Advanced Science Institute, Saitama, Japan

Brassinosteroids (BRs) are a unique class of plant hormones widely distributed throughout the plant kingdom that are essential for normal plant development and growth. Plants defective in BR biosynthesis or perception display characteristic phenotypes including a dark green, dwarf stature, delayed flowering, reduced male fertility and photomorphogenesis in the dark.

Genetic and biochemical approaches indicate that plant GSK3/SHAGGY-like kinases (GSKs) are involved in various biological processes including hormone signalling. Plants possess a small family of these proteins. Research

has linked group II GSKs to BR signalling, however there is evidence that additional GSKs are involved in regulation of BR signal transduction.

Data are presented that link a novel GSK3/SHAGGY-like kinase from *Arabidopsis thaliana* (ASK3) to BR signalling. Plants overexpressing ASK3 exhibit a phenotype with striking similarities to mutants of the BR receptor BRI1, namely severe dwarfism, dark green, curled leaves, a shortened hypocotyl, and partial de-etiolation. ASK3 overexpressors are insensitive to exogenously applied epibrassinolide and have increased levels of bioactive BRs, which are characteristics of plants with impaired BR signalling. In contrast, plants overexpressing a kinase-dead version of ASK3 do not exhibit an obvious phenotype. BEH2, a BES1/BZR1-like transcription factor involved in BR signalling, was isolated as an ASK3 interaction partner in a yeast two-hybrid screen. Both proteins are localised to the nucleus and BEH2 can be phosphorylated by ASK3 *in vitro* and *in vivo*. Overall, these data indicate that ASK3 is a novel component of BR signalling, targeting the transcription factor BEH2.

P542 Arabidopsis RPT2a, a subunit of 19S proteasome that negatively regulates endoreduplication and confers leaf size control

Kaori Sako, Yutaka Sonoda, Yuko Maki, Hiroko Yamamoto, Junji Yamaguchi

Graduate Schl. Life Science, Hokkaido Univ., Sapporo, Japan

The ubiquitin/26S proteasome pathway plays a central role in the degradation of short-lived regulatory proteins to control many cellular events. To further understand this pathway, we focused on the RPT2 subunit of the 26S proteasome regulatory particle. The Arabidopsis genome contains two genes, *AtRPT2a* and *AtRPT2b*, which encode paralog molecules of RPT2 subunit with a difference of only three amino acids in the protein sequence. Both genes showed similar mRNA accumulation patterns. However, the *rpt2a* mutant showed a specific phenotype of enlarged leaves caused by increased cell size in correlation with increased ploidy. Detailed analyses revealed that cell expansion is increased in the *rpt2a* mutant by extended endoreduplication early in leaf development. Transcription of genes encoding cell cycle-related components for DNA replication licensing and G2/M phase was also promoted in the *rpt2a* mutant, suggesting that extended endoreduplication was caused by increased DNA replication and disrupted regulation of the G2/M check point at the proliferation stage of leaf development.

P543 Isolation of a novel RING-type ubiquitin ligase SSV1 that regulates carbon/nitrogen response at early post-germinative growth stage in *Arabidopsis thaliana*

Takeo Sato, Shugo Maekawa, Yutaka Sonoda, Junji Yamaguchi

Graduate Schl. Life Science, Hokkaido Univ., Sapporo, Japan

Carbon and nitrogen availability is one of the most important factors regulating plant development. To utilize limited resources of carbon and nitrogen efficiently, plants are able to sense and respond to the balance of carbon (C) and nitrogen (N) metabolites, called the C/N response.

To clarify mechanisms involved in the C/N response in higher plants, we had isolated and characterized a mutant named *ssv1-D* (*super survival 1-D*), which was resistant to severe C/N nutrition stress (high-sugar and low-nitrogen) conditions. The mutant was due to overexpression of the *SSV1* gene and the expressions of C and/or N-regulated genes were not regulated precisely in the *ssv1-D* mutant. *SSV1* loss of function mutant was hypersensitive to change of C/N conditions. The *SSV1* gene encodes RING type ubiquitin ligase (E3) and the translated protein showed E3 activity *in vitro*. Furthermore, removal of the ubiquitin ligase activity from the overexpressed gene resulted in loss of the mutant phenotype. These results suggest that the SSV1 regulates C/N response via ubiquitin cascade-26S proteasome system in higher plants. Detailed characterization of the mutant will be reported.

P544 Molecular characterization of EIN2, a central element in plant hormone signalling

Silke Allekotte, Jan Voet van Vormizeele, Nicole Voet van Vormizeele, Georg Groth

Department of Biology, Plant Biochemistry, Heinrich-Heine University, Düsseldorf, Germany

Reverse genetics has identified the membrane protein Ethylene Insensitive 2 (EIN2) as a central component of ethylene signalling in Arabidopsis.[1] Sequence analysis suggests a bipartite structure of EIN2 consisting of a hydrophobic amino-terminal domain (amino acids 1-461) which was shown to constitutively activate ethylene responses in mutant plants and a predominately hydrophilic carboxyl-terminal region (amino acids 462-1294) which does not show homology to any known protein. Similarity of the amino-terminal domain of EIN2 to a mammalian family of metal-ion transporters (NRAMP) suggested that EIN2 might function as sensor or even as transporter of divalent cations.[1] However, neither of these functions nor the underlying molecular mechanism of signal transfer to or from EIN2 have been resolved yet. We have cloned and expressed the carboxyl-terminal part of EIN2 from *Arabidopsis thaliana* in the enterobacterium E. coli. Overexpressed recombinant EIN2 was purified to homogeneity from extracts of the *E. coli* cells by metal-chelate affinity chromatography and gel filtration. A single band on SDS-PAGE at an apparent molecular weight of 98 kDa confirmed purity and homogeneity of the recombinant protein. The purified EIN2 was characterized by biochemical and biophysical techniques. Fluorescence spectroscopy, taking advantage of the endogenous tryptophan residues in the recombinant EIN2 and isothermal titration calorimetry, revealed that EIN2 binds 3-4 calcium ions in its carboxyl-terminal domain with dissociation constants of 7 µM and 410-860 µM, respectively. CD spectroscopy revealed that binding of the divalent ion is associated with a substantial conformational change in the EIN2 protein. Our results imply a specific interaction of the carboxyl-terminal domain of EIN2 with calcium and suggest that calcium might play a role in signaling to and/or from EIN2.

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker, JR (1999). EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. Science 284: 2148–52.

P545 Arabidopsis RACK1 proteins are negative regulators of ABA signalling in seed germination and early seedling development

Jianjun Guo1, Junbi Wang1,2, Wei-Dong Huang2, Jiansheng Liang3, Jin-Gui Chen1

1Department of Botany University of British Columbia, Vancouver, (BC V6T 1Z4), Canada, 2College of Food Science and Nutritional Engineering China Agricultural University, Beijing (100083), China, 3College of Bioscience and Biotechnology Yangzhou University, Yangzhou (225009), China

Abscisic acid (ABA) is one of the most important plant hormones regulating plant stress responses as well as many aspects of plant development. We previously showed that loss-of-function of *RACK1A (Receptor for Activated C Kinase 1),* one of the three *RACK1* homologous genes in Arabidopsis, rendered ABA hypersensitivity. Here, we provide genetic and molecular evidence that all three RACK1 proteins are negative regulators of ABA signalling in seed germination and early seedling development. We found that loss-of-function mutations in *RACK1B* and *RACK1C* enhanced the ABA hypersensitivity of *rack1a* mutant in the ABA inhibition of seed germination, cotyledon greening and root growth. Moreover, plants overexpressing *RACK1A* displayed ABA insensitivity. Consistent with their proposed roles in seed germination and early seedling development, all three *RACK1* genes were expressed in imbibed, germinating and germinated seeds. Expression analysis indicated that *ABI1* and *ABI2*, which encode two key negative regulators of ABA signalling that act to reset ABA responses, displayed much lower response to ABA in the *rack1a rack1b* double mutant seedlings, implying that *RACK1* may be required for the proper desensitization of the triggered ABA signalling cascade. Furthermore, ABA induction of a group of potential physical interacting partners of *RACK1* (identified by the Arabidopsis Interaction Reviewer) which are known to be involved in ABA or stress signalling were misregulated in the *rack1a rack1b* double mutants. Taken together, our findings provide compelling evidence that RACK1 proteins are critical negative regulators of ABA signalling.

P546 The role of the *Gibberellin 20-Oxidase* gene family in Arabidopsis development

Andrew Plackett1,2, Nieves Fernandez-Garcia3, Steven Thomas1, Peter Hedden1, Andy Phillips1, Zoe Wilson2

1Rothamsted Research, U.K., 2University of Nottingham, UK, 3Universitario de Espinado, Spain

The *Gibberellin 20-oxidase (GA20ox)* gene family in *Arabidopsis thaliana* comprises five closely related members distributed throughout the genome. Each GA20ox enzyme catalyses a series of intermediate oxidation reactions during the biosynthesis of the phytohormone gibberellin (GA), which plays a role in many developmental processes throughout the plant lifecycle. Evidence suggests that *GA20ox* activity may be rate limiting for GA biosynthesis, and that the individual members of the gene family are under tight regulation with overlapping but distinct profiles.

Previous work by our group identified and characterised the effect of knockout insertion mutations in the most highly expressed members of this family, *GA20ox1* and *GA20ox2*, in the Columbia ecotype. The double mutant has a semi-dwarf phenotype, with delayed flowering and commonly displaying male sterility within the first ten flowers produced. However, this phenotype is far less severe than the *ga1-3* biosynthetic mutant, which is considered to be almost entirely GA-deficient. A broad-scale quantitative RT-PCR analysis of the *GA20ox* gene family implies that *GA20ox3* is also a highly expressed member of this gene family and may have particular importance to reproductive development.

A knockout TILLING mutation in *GA20ox3* has now been isolated and is currently being characterised in association with *GA20ox1* and *GA20ox2*. The triple mutant displays a far more severe phenotype than *ga20ox1ga20ox2* at most stages of development, with a close resemblance to *ga1-3* but with differences that imply minor roles for the remaining *GA20ox* genes. In particular the *ga20ox1ga20ox2ga20ox3* mutant displays full male sterility, apparently due to defects in stamen elongation and anther dehiscence. Work is continuing to characterise the relationships between the different *GA20ox* genes in various aspects of plant development, including the incorporation of mutations in *GA20ox4* and *GA20ox5* and a close analysis of the expression profile of each gene via transgenic GUS reporter lines currently under development.

P547 Indole-3-acetaldoxime dependent auxin biosynthesis in Arabidopsis

Satoko Sugawara, Yuji Kamiya1, Yusuke Jikumaru, Atsushi Hanada, Shojiro Hishiyama2, Takeshi Nishimura3, Yunde Zhao4, Tomokazu Koshiba, Hiroyuki Kasahara

1RIKEN PSC, Yokohama, Japan, 2Forestry & Forest Product Res. Inst. Ibaraki, Japan, 3Tokyo Met. Univ., Hachioji, Japan, 4UC San Diego, La Jolla, CA, USA

Auxins are hormones that regulate many aspects of plant growth and development. Indole-3-acetaldoxime (IAOx) has been proposed to be a key intermediate in the synthesis of indole acetic acid (IAA) and several other indolic compounds. Genetic studies of IAA biosynthesis in Arabidopsis have suggested that 2 distinct pathways involving the *CYP79B* or *YUCCA (YUC)* genes may contribute to IAOx synthesis and that several pathways are also involved in the conversion of IAOx to IAA. We present the biochemical dissection of IAOx biosynthesis and metabolism in plants by analyzing IAA biosynthesis intermediates. We demonstrated that the majority of IAOx is produced by *CYP79B* genes in Arabidopsis because IAOx production was abolished in *CYP79B* deficient mutants. IAOx was not detected from rice, maize, and tobacco, which do not have apparent *CYP79B* orthologues. IAOx levels were not altered in the *yuc1 yuc2 yuc4 yuc6* quadruple mutants,

suggesting that the *YUC* gene family probably does not contribute to IAOx synthesis. We determined the pathway for conversion of IAOx to IAA by identifying 2 intermediates, indole-3-acetamide (IAM) and indole-3-acetonitrile (IAN), in Arabidopsis. When $^{13}C_6$-labeled IAOx was fed to *CYP79B*-deficient mutants, $^{13}C_6$ atoms were efficiently incorporated to IAM, IAN, and IAA. This biochemical evidence indicates that IAOx-dependent IAA biosynthesis, which involves IAM and IAN as intermediates, is not a common but a species-specific pathway in plants; thus IAA biosynthesis may differ among plant species.

P548 A role for KLU in seed size control

Nikolai M Adamski1, Elena Anastasiou2, Sven Eriksson1, Carmel O'Neill1, Michael Lenhard1

1John-Innes-Centre, Norwich, UK, 2Institut fur Biologie III der Universitat Freiburg, Freiburg, Germany

Seed size is both an important ecological and agronomical factor for plant development. Enhanced seed size confers significant advantages to the germinating seedlings, like a higher amount of resources, a higher rate of growth or a higher rate of seedling establishment. In addition, seed size is an interesting trait for crop improvement.

The cytochrome P450 *CYP78A5/KLUH (KLU)* was recently shown to control plant organ growth, most likely by generating a novel growth stimulating signal. Here, we show that *KLU* also has a specific, positive effect on final seed size.

Loss-of-function mutants of *KLU* produce seeds with a reduced size compared to wild type. By contrast, gain-of-function plants give rise to bigger seeds. Using genetic grafting, *KLU* activity was shown to regulate seed size in a local, ovule-specific manner. Reciprocal crosses showed that *KLU* acts via the maternal sporophyte to define seed size. Double mutant analysis demonstrated that *KLU* acts independently of other genes, like *AP2* or *ARF2*, that are known to regulate seed size maternally.

Together, these results indicate the potential of *KLU* to be used for crop improvement regarding seed size.

P549 An intracellular ABA receptor regulates ABA signaling through direct inhibition of clade A PP2Cs

Julia Santiago1, Americo Rodrigues1, Angela Saez1, Silvia Rubio1, Regina Antoni1, Lesia Rodriguez1, Sang-Youl Park2, Florine Dupeux3, Jose Marquez3, Sean Cutler2, Pedro Rodriguez1

1IBMCP, Valencia, Spain, 2University of California, Riverside, USA, 3EMBL, Grenoble, France

Abscisic acid (ABA) is a key phytohormone to cope with environmental stress and regulation of plant development. Clade A protein phosphatases type-2C (PP2Cs), such as HAB1, are key negative regulators of ABA signaling in Arabidopsis. To gain further insight into regulation of HAB1 function by ABA, we have screened for HAB1-interacting partners through a yeast two-hybrid approach. Three proteins were identified, PYL5, PYL6 and PYL8, which belong to a 14-member subfamily of the Bet v1-like superfamily. HAB1-PYL5 interaction was confirmed using BiFC and co-immunoprecipitation assays. PYL5-overexpression led to global enhanced response to ABA, whereas an opposite phenotype had been reported for HAB1-overexpressing plants.

F2 plants that overexpressed both HAB1 and PYL5 showed enhanced response to ABA, which indicates that PYL5 antagonizes HAB1 function. PYL5 and other members of its protein family inhibited HAB1, ABI1 and ABI2 phosphatase activity in an ABA-dependent manner. Isothermal titration calorimetry revealed saturable binding of PYL5 to (+)ABA, Kd=1.1 uM.

Our work indicates that PYL5 is an intracellular ABA receptor that activates ABA signaling through direct inhibition of clade A PP2Cs

P550 A novel point mutation in a *Poly (A) polymerase* inversely affects the size of leaves and flowers in *Arabidopsis thaliana*

Son Lang Vi, Nicola Stacey, Michael Lenhard

John Innes Centre, Norwich, UK

Different structures in an organism grow to different final sizes depending on their identity. For example in Arabidopsis, leaves are much larger than petals and sepals, despite representing homologous organs. To achieve this, plants may employ organ-specific size regulators, such as *BIG PETAL (BPE)*.

However, most of the reported size regulators so far are not organ-specific, and mutations in these generic regulators affect both leaves and petals in a similar way, i.e. either enlarging or reducing the sizes of both. What is unknown at present is how generic size regulation can be modified in an organ-specific manner.

Here we describe a novel recessive EMS mutation that leads to smaller leaves but bigger petals than wild-type. The phenotype suggests that this putative size regulator is generic, i.e. acts in both organs, but affects leaves and flowers in an opposite manner.

This phenotype thus provides a chance to gain insight into the above question. Growth parameter measurements indicate that the bigger size in petals is due to a prolonged growth period, leading to more and bigger cells.

Surprisingly, the mutation affects a nuclear *Poly(A) polymerase (PAP)*, one of four *PAPs* in Arabidopsis, which participate in mRNA 3' end processing by adding poly(A)-tails to mRNAs. Analyzing T-DNA insertion lines for all *PAPs* suggests that the phenotype is not only specific to this *PAP*, but also to this point mutation.

We present a hypothesis to explain how a mutation in what appears to be a house-keeping enzyme can have specific growth phenotypes, and why the size effects are opposite between leaves and petals.

P551 The extracellular EXO protein mediates cell expansion in Arabidopsis

Florian Schröder1, Janina Lisso1, Carsten Müssig2

1Max Planck Institute of Molecular Plant Physiology, Am M¸hlenberg1, 14476 Golm, Germany, 2GoFORSYS, Universiat Potsdam, c/o Max Planck Institute of Molecular Plant Physiology, Am Muhlenberg1, 14476 Golm, Germany

The *EXO (EXORDIUM)* gene was identified as a brassinosteroid (BR)-regulated gene. It is part of a gene family with eight members in Arabidopsis. The EXO protein is an extracellular protein that mediates BR-induced cell expansion in above-ground organs. The *exo* loss of function mutant showed diminished leaf and root growth. Light and scanning electron microscopy analyses revealed that impaired leaf growth is due to reduced cell expansion. Epidermis, palisade, and spongy parenchyma cells were smaller in comparison to the wild-type. The *exo* mutant showed reduced brassinolide-induced cotyledon and hypocotyl growth. In contrast, *exo* roots were significantly more sensitive to the inhibitory effect of synthetic brassinolide. Apart from reduced growth, *exo* did not show severe morphological abnormalities. Gene expression studies suggest that EXO does not control BR-levels or BR-sensitivity in the shoot. EXO presumably coordinates BR-responses with environmental or developmental signals. Expression of the *EXL1 (EXO-LIKE1), EXL3*, and *EXL5* genes also depends on BR, and preliminary data suggest that these genes control growth as well. A diverse set of transgenic plants and mutants was established. The resources are used to determine the relationships of the *EXO, EXL1, EXL3*, and *EXL5* genes, to identify interacting proteins, to test the dependency of BR-responses on the genes, and to analyze the molecular mechanisms of EXO/EXL-promoted growth. The project is expected to provide novel insights into BR-dependent growth control in Arabidopsis. Similar proteins are encoded in crop genomes. The analysis of the EXO and EXL proteins has the potential to reveal a novel way to control crop growth.

P552 Gibberellin biosynthesis and action in Arabidopsis roots

Richard Barker1, Stephen Thomas1, Peter Hedden1, Malcolm Bennett2

1Rothamsted Researcg, Herts, UK, 2The University of Nottingham

Gibberellins (GAs) are growth promoting hormones that have an essential role in regulating plant growth. Recent work has demonstrated that bioactive GAs regulate plant growth and development by degrading the growth repressing DELLA proteins, and mutations within genes that encode certain biosynthetic enzymes have a dwarf stature and display reduced root elongation. In the Arabidopsis root, GA-dependant DELLA degradation is required only within the endodermis in order to promote local tissue and ultimately whole organ growth. Interestingly, the expression of GA biosynthetic genes occurs in distinctly different cell layers, suggesting that the movement of bioactive GAs or their precursors may occur.

The aim of this project is to dissect the various root tissues and stages of development in order to determine the precise locations in which GA biosynthesis and signalling occur to promote Arabidopsis root growth. I have targeted the expression of genes encoding GA-inactivating enzymes, which specifically act on C20-GA precursors or active C19-GAs, to 6 specific cell types within the root using tissue specific promoters. Inhibition of growth through cell-type specific inactivation of C20-GAs or active C19-GAs will be indicative of sites of biosynthesis or perception/biosynthesis, respectively. In addition, sites of biosynthesis and action are being further investigated by attempting to rescue the root elongation phenotype of mutants lacking GA receptors or key biosynthetic enzymes using cell-type-specific expression of functional versions of the mutant genes. The GA-inactivating enzymes AtGA2ox2 and AtGA2ox7 have been expressed as N-terminal YFP fusion proteins and their functions confirmed in *in vitro* assays. Subsequently, all constructs for expression in 6 different cell types have been made, sequenced and transformed into wild-type or mutant Arabidopsis plants. Transgenic T1 plants are currently being selected with the aim of isolating homozygous lines in the T3 generation. These will be characterised using confocal microscopy and will subsequently undergo detailed analysis of root growth using semi-automated kinematic computer programs in conjunction with time lapse photography.

P553 Putative phosphorylation sites of PIN3 are critical for its auxin efflux activity in Arabidopsis root hairs

Anindya Ganguly, Sang Ho Lee, Hyung-Taeg Cho

School of Biological sciences, Seoul National University, Seoul 151-742, Korea

The PIN protein family in Arabidopsis acts as auxin efflux carriers, which play a major role in maintaining local auxin gradients throughout the plant body. PIN proteins have their own cell type-specific expression pattern and are highly dynamic and polar in their localization within a given cell type. Protein kinases seem to modulate the PIN proteins by direct phosphorylation in the central cytoplasmic loop of PIN proteins. PIN3 has been shown to mediate auxin efflux both *in planta* and in tobacco BY2 cells. Here, we expressed PIN3 (PIN3ox) and PIN4 (PIN4ox) specifically in the Arabidopsis root hair cells using the root hair-specific expansinA7 promoter (pE7). PIN3ox in root hair cells greatly suppressed root hair growth most likely due to facilitation of auxin efflux. PIN3ox and PIN4ox showed differential root hair-suppressing strengths with longer root hairs by PIN4ox than by PIN3ox, although both GFP-fused proteins were correctly targeted to the plasma membrane. To get an insight into the molecular cues of the PIN proteins for their molecular activity, we mutated several putative phosphorylation residues of PIN3, expressed the mutant proteins under pE7, and checked the root hair phenotype. Some of the mutations caused restoration of the hair growth almost to the wild-type level, indicating that those putative phosphorylation residues are required for the auxin efflux activity of the PIN3 protein. Further detailed analysis of the role of the putative phosphorylation sites and the cytosolic domain of PINs will be helpful to understand the molecular mechanism of PIN function.

This study was supported by grants from KOSEF, CFGC, BioGreen 21, and EBNCRC.

P554 AtDOF6, an Arabidopsis DOF transcription factor putatively involved in the regulation of seed germination

Paloma Rueda, Cristina Barrero, Pilar Carbonero, Luis Oñate-Sánchez

Centro de Biotecnologìa y Genumica de Plantas-Universidad Politecnica de Madrid, ETSI Agrunomos, 28040 Madrid, Spain

DOF proteins (DNA binding with One Finger) are transcription factors (TFs) that play diverse roles in gene regulation of plant-specific processes. In barley (*Hordeum vulgare*), 26 different DOFs have been described. Four of them (BPBF, SAD, HvDOF19 and HvDOF17) have been shown to play a role, not only in seed storage protein expression during seed maturation, but also in the regulation of hydrolase gene expression in the germinating aleurone cells.[1-6]

We have analyzed Dof gene expression by RTqPCR in Arabidopsis germinating seeds, and have identified *AtDof6* as an early and highly induced gene. Its expression profile precedes that of genes putatively involved in reserve mobilization (lipases, proteases) and in cell-wall modification (xyloglucan-endotransglycosylases, expansins). Since no KO mutants for this gene could be found in public data-bases, we have generated gain of function and amiRNA transgenic plants, in order to study the possible regulatory role of *AtDof6* within this physiological process.

Phylogenomic approaches have revealed the presence of conserved *cis* elements containing DOF-binding motives (5'T/A-AAAG 3') in hydrolase gene promoters in Arabidopsis and closely related Brassicaceae. We are performing transient expression assays to further characterize the function of AtDof6 in the transcriptional control through these motifs. To identify AtDof6 interacting partners, we are performing a yeast-2-hybrid screening with an Arabidopsis normalized TF library.

1 Diaz, I., *et al* (2005) Plant J 42, 652-662
2 Isabel-LaMoneda, I., *et al* (2003) Plant J 33, 329-340
3 Mena, M., *et al* (2002) Plant Physiol 130, 111-119
4 Mena, M., *et al* (1998) Plant J 16, 53-62
5 Moreno-Risueno, M.A., *et al* (2007a) Plant J 51, 352-365
6 Moreno-Risueno, M.A., *et al* (2007b) Mol Genet Genomics 277, 379-390

P555 The molecular mechanism of growth repression by BIG BROTHER

Lena Stransfeld, Michael Lenhard

John Innes Centre, Norwich, UK

The growth of an organ, i.e. biomass accumulation, is controlled by species-specific genetic mechanisms. The different pathways that promote or restrain cell and organ growth must be precisely coordinated. For organs to reach their specific size, growth must be terminated after a certain time.

One of the central negative regulators of organ growth is the *BIG BROTHER (BB)* gene. *BB* limits the growth of floral organs and the stem in a strictly dosage-dependent manner by restricting the period of proliferative growth. The *BB* gene encodes an E3 ubiquitin ligase, which contains a RING-finger domain. The E3 activity of BB protein suggests that BB limits organ size by marking crucial growth stimulators for proteasomal degradation. Since BB functions independently of the major phytohormones and known organ growth promoter, such as *JAG* and *ANT*, identification of its substrates may uncover essential novel activators of plant growth.

By employing the repressed transactivator system (RTA), a member of the homeobox gene family was identified that specifically interacts with BB. Our results suggest that BB restricts organ growth by marking key transcription factors for degradation.

P556 Active CLV3 is an arabinosylated glycopeptide

Kentaro Ohyama, Hidefumi Shinohara, Mari Ogawa-Ohnishi, Yoshikatsu Matsubayashi

Graduate School of Bio-agricultural Science, Nagoya University, Nagoya, Japan

Plants continuously produce organs from the self-renewing shoot apical meristem (SAM). A receptor kinase CLV1 and a secreted peptide CLV3 are key components of the regulatory network controlling stem cell renewal and differentiation in Arabidopsis. CLV3 belongs to the CLV3/ESR (CLE) family of peptides that contain a short conserved domain (CLE domain) at or near the C-terminus. Several lines of evidence suggest that the CLE domain, which is the only region with similarity among CLE family peptides, is the functional domain of CLV3. However, we have reservations about the proposed structure for the CLV3 functional form, mainly because exogenous application of the 12-amino-acid CLE domain peptide does not fully rescue *clv3* mutant phenotypes at physiologically relevant concentrations. In addition, although the CLE subfamily (such as genes *CLE1* to *CLE7*) can almost fully complement phenotypes of *clv3* mutant under the *CLV3* promoter, exogenous application of the corresponding 12-amino-acid CLE domain peptides does not rescue *clv3* phenotypes. We thus assume that mature functional form of CLV3 may have undergone as-yet undiscovered posttranslational modifications critical for their function. Here, we show, by nano-LC-MS/MS analysis of apoplastic peptides of Arabidopsis plants overexpressing CLV3, that active mature CLV3 is a 13-amino-acid arabinosylated glycopeptide. We treated *clv3-2* mutant seedlings with purified CLV3 glycopeptide and observed that the *clv3-2* SAM treated with CLV3 at 30 nM were substantially reduced in size comparable to wild-type levels. In contrast, synthetic peptide devoid of arabinose showed only weak activity, indicating that the arabinose chain of CLV3 is critical for full activity.

We also analyzed the binding affinity of CLV3 glycopeptide to CLV1 receptor kinase and confirmed that CLV3 glycopeptide interacted with the CLV1 ectodomain far more strongly than the non-arabinosylated forms. Collectively, we propose that active mature CLV3 is an arabinosylated glycopeptide.

Ohyama *et al* Nature Chem. Biol. (2009) in press.

P557 CES, a novel basic helix-loop-helix transcription factor regulating brassinosteroid responses and homeostasis

Wilfried Rozhon1, Mamoona Khan1, Sigrid Husar1, Shozo Fujioka2, Gerhard Adam3, Christian Luschnig3, Tobias Sieberer1, Brigitte Poppenberger1,3,4

1Max F. Perutz Laboratories, University of Vienna, Dr. Bohrgasse 9, Vienna, Austria, 2Discovery Research Institute, RIKEN, Wako-shi, Saitama, Japan, 3Department of Applied Plant Sciences and Plant Biotechnology, BOKU - University of Natural Resources and Applied Life Sciences, Vienna, Austria, 4Center for Novel Agricultural Products, University of York, York, UK

Brassinosteroids (BRs) comprise a class of over 40 polyhydroxylated sterol derivatives that appear to be ubiquitously distributed throughout the plant kingdom. Brassinosteroids regulate expression of numerous genes, impact on the activity of complex metabolic pathways, contribute to regulation of photomorphogenesis, cell elongation, division and differentiation including vascular tissue development and pollen tube growth.

Here we describe the identification and characterisation of the basic helix-loop-helix transcription factor CESTA (CES), a novel regulator of BR biosynthesis gene expression. *ces-D* was identified as a dominant activation-tagged mutant of *Arabidopsis thaliana* that displays constitutive BR responses. The transcript abundance of BR biosynthesis genes as well as the levels of bioactive BRs were elevated in this mutant.

In order to isolate interaction partners of CES a yeast two-hybrid interaction screen was performed. This revealed that CES can homodimerise and heterodimerise with closely related bHLH transcription factors, which could be confirmed by bimolecular fluorescence complementation experiments. Interestingly, treatment with brassinolide caused rapid re-localisation of CES to nuclear speckles.

Genes potentially targeted by CES were recognised in a microarray experiment. Electro mobility shift assays revealed that CES binds to G-boxes in the promoters of such genes and might thereby modulate the transcription activity of these promoters.

Taken together these data provide strong indications that CES acts as a novel component in BR signalling modulating BR responses and impacting on BR homeostasis by controlling transcription of rate-limiting biosynthesis genes.

P558 Methyl jasmonate affects adventitious rooting in *Arabidopsis thaliana* plants and thin cell layers

Laura Fattorini1, Giuseppina Falasca1, Federica Della Rovere1, Catherine Bellini2, Maria Maddalena Altamura1

1Dipartimento di Biologia Vegetale, Sapienza Universite di Roma, Rome, Italy, 2UPSC, Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, Umea, Sweden

Adventitious root (AR) formation is affected by numerous endogenous/exogenous factors, but its hormonal and genetic control is not yet fully understood. It is known that exogenous auxins stimulate adventitious rooting *in planta* and in *in vitro* cultured explants. By contrast, little information is available about the effect of jasmonates on this process. These compounds, including jasmonic acid (JA) and methyl jasmonate (MeJA), are common in plants, with roles in development and defense. In plantlets grown *in vitro* under long-days of the most common *Arabidopsis thaliana* ecotypes, one/two ARs are usually formed at the hypocotyl/primary root transition zone. However, the number and the localization of ARs can change depending on the experimental growth conditions, e.g. an increase occurs under darkness and in the presence of 0.5% sucrose [Takahashi *et al*, J Plant Res (2003), 116: 83]. Under the latter conditions, we added MeJA, at 0, 0.01, 0.1, 10 µM, to the medium and evaluated root productivity in Arabidopsis mutants blocked in two different steps of JA biosynthesis, i.e. in *dde2-2* and *opr3* mutants, and in their wild types. Moreover, for studying the interaction between jasmonates and auxins, thin cell layers (TCLs) were excised from the stem internodes of these mutants, and of the wild types, and cultured *in vitro* in presence/absence of MeJA (0-10 µM), either under hormone-free conditions or with the optimal exogenous hormones to induce rooting from Arabidopsis TCLs [IBA 10 µM plus Kin 0.1 µM, Falasca et al, Plant Cell Rep (2004), 23: 17]. We also investigated *in planta* AR formation in the *sur2-1* mutant, which is known to accumulate endogenous IAA and overproduce ARs from the hypocotyl [Delarue *et al*, Plant Journal (1998), 14: 603]. All together, the results suggest an involvement of jasmonates in adventitious rooting, and an interaction with auxin.

P559 Fruit growth in Arabidopsis occurs via DELLA-dependent and DELLA-independent GA responses

Sara Fuentes1, Liz Alvey2, Karin Ljung3, Karim Sorefan1, Nicholas P Harberd2,4, Lars Østergaard1

1Department of Crop Genetics, John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK, 2Department of Cell and Developmental Biology, John Innes Centre, Colney Lane, Norwich, NR4 7UH, UK, 3Department of Forest Genetics and Plant Physiology, Umea Plant Science Centre, S-901 83 Umea, Sweden, 4Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK

Like most developmental processes, fruit development is regulated by complex hormonal networks. Previous studies have shown that the phytohormone gibberellic acid (GA) promotes growth by inducing degradation of the growth-repressing DELLA proteins; however, the extent to which DELLA proteins contribute to GA-mediated gynoecium and fruit development remains to be clarified. In order to achieve this aim, an in-depth characterisation of the role of DELLA proteins in fruit-set and growth was carried out. We show that DELLA proteins are not only key regulators of reproductive organ size but are also involved in the control of wider aspects of plant sexual reproduction. In addition, we have confirmed that the facultative parthenocarpy observed in *della* mutants can be directly attributed to the constitutive activation of GA signalling. Our systematic genetic analysis shows that Arabidopsis

DELLA proteins RGA, GAI, RGL1 and RGL2 jointly repress pistil development in the absence of fertilisation. Finally, we have uncovered a novel DELLA-independent GA response during fruit development. Taken together, our results show that DELLA proteins are key regulators of gynoecium and fruit development. Moreover, control of GA-signalling during fruit development is likely to rely on additional levels of complexity as suggested by the existence of a DELLA-independent GA response.

P560 Differential expression of *Arabidopsis thaliana* sucrose phosphate synthase and sucrose phosphate phosphatase encoding genes

Cristóbal Girón, Beatriz Castrillo, Federico Valverde, Mª Teresa Ruiz, José Mª Romero

Instituto de Bioquimica Vegetal y Fotosintesis, Seville, Spain

Transport of sucrose from source to sink tissues provides energy and carbon source for growth and development. Sucrose not only has a role as a signalling molecule in metabolism and development, but it has also been postulated to have a role in other processes such as flowering. The final steps of the sucrose synthesis pathway consist of the sequential action of the enzymes sucrose-phosphate synthase (SPS) and sucrose-phosphate phosphatase (SPP). SPS is the most tightly regulated of both enzymes, which are proposed to form a temporary complex.

We determined the absolute steady-state mRNA levels of the four *SPS* and the four *SPP* encoding genes present in *Arabidopsis thaliana* by real-time RT-PCR. In the organs analyzed, *SPS5.1* is the one expressed at highest levels, followed by *SPS4* in aerial organs and by *SPS5.2* in roots. Among the *SPPs* genes *SPP1*, and to a lesser extent *SPP2*, are the ones showing highest levels of expression in aerial organs, while in roots *SPP4* is the most highly expressed. These data suggest an organ-specific pattern of expression for both gene families. In leaves of plants grown in long days, the mRNA level of the most highly expressed genes (*SPS5.1-SPS4 and SPP1-SPP2*) exhibit circadian oscillation, with a minimum at 8-12 ZT and a maximum at dusk. The less expressed genes do not show a clear oscillatory pattern. These data suggest a circadian control of the sucrose synthesis, at least in aerial organs.

CONSTANS (CO) regulates flowering through the integrators *FT* and *SOC1*. We carried out similar expression analysis of *SPSs* and *SPPs* in the leaves of *co-2* and 35S::CO, mutant and overexpressor lines of *CO*, respectively. In these plants, only the expression of *SPS4* seems to be affected, its mRNA being significantly increased in the 35S::CO line. In its response to CO, *SPS4* behaves in a similar way to *GBSSI* (Granule-Bound Starch Synthase). These results suggest that, in order to promote flowering, CO may alter carbon fluxes by modifying the expression of key enzymes involved in starch and sugar metabolism.

P561 DELLA-mediated transcriptional control of GA-responsive growth and development in Arabidopsis

Anne Grønlund1, Jane Griffiths1, Aakriti Wanchoo1, Michael Wilson2, Tara Holman2, Malcolm Bennett2, Andy Phillips1, Peter Hedden1, Stephen Thomas1

1Centre for Crop Genetic Improvement, Rothamsted Research, Harpenden. AL5 2JQ, 2CPIB, University of Nottingham, Sutton Bonington. LE12 5RD

In recent years there have been major advances in our understanding of how the plant hormone gibberellin (GA) promotes essential processes of plant development. DELLA proteins (DELLAs) are central to the GA signalling cascade where they act as repressors of GA-responsive growth. GA signalling relieves the repression exerted through DELLAs by targeting their degradation. DELLA proteins are nuclear localised and due to their ability to regulate the expression of GA-responsive genes it is suggested they act as transcriptional regulators. However, a canonical DNA-binding domain is absent in DELLAs and it is therefore likely that they regulate the expression of GA-responsive genes through interactions with other transcription factors. This is supported by the recent findings that DELLAs interact with PIF transcription factors to control light-mediated hypocotyls elongation.

This work is aimed at improving our understanding of the role of DELLAs and their downstream target genes in regulating GA-responsive growth in Arabidopsis. Microarray experiments have been performed on GA-deficient Arabidopsis mutants, resulting in the identification of many novel GA-responsive genes that are potential primary targets of DELLAs. A reverse genetics-based strategy is subsequently being employed to uncover the role of these potential DELLA target genes in regulating GA-responsive growth. It is known that many of the genes encoding GA signalling components that interact with DELLAs are also transcriptionally regulated by the GA-signalling pathway. Therefore, in combination with the microarray studies, we have performed yeast two-hybrid screens, with the aim of identifying novel DELLA-interacting proteins that are responsible for regulating the expression of GA-responsive genes. These screens have identified novel DELLA interactors, whose genes were also demonstrated to be GA-responsive in the microarray experiments. The role of the novel DELLA interactors is currently being investigated.

P562 Flavonol 3-O-glycosylation is required for flavonol biosynthesis in Arabidopsis

Yin Ruohe, Messner Burkhard, Anton R Schaffner

Institute of Biochemical Plant Pathology, Helmholtz Zentrum Munchen, 85764 Neuherberg, Germany

Glycosyltransferases transfer sugar moieties onto lipophilic small molecules to alter their properties like solubility, bioactivity or access to membrane transport. Flavonols like kaempferol and quercetin are phenylpropanoid-derived secondary metabolites with multiple roles in plant development and defence. In Arabidopsis,

the first step of flavonol glycosylation occurs at the 3-*O*-position, which can be catalyzed by UGT78D1 and UGT78D2. Flavonols are maintained at wild-type levels in *ugt78d1* and *ugt78d2* single mutants due to the compensatory effect by the other, remaining flavonol glycosyltransferases. In contrast, *ugt78d1 ugt78d2* double mutant plants exhibit significantly reduced flavonols in leaves and flowers. This suggests that these two enzymes are mainly responsible for flavonol 3-*O*-glycosylation in these organs and that 3-*O*-glycosylation by these two enzymes is required for flavonol biosynthesis. Expression studies of UGT78D1 and UGT78D2 based on promoter-reporter lines and publicly available data are in agreement with this organ specificity. According to these results, the loss of the initial 3-*O*-glycosylation represses the flavonol biosynthesis, probably to avoid the accumulation of toxic flavonol aglyca. Interestingly, the transcription and enzyme activity of PAL – acting upstream as a known committed step into the phenylpropanoid pathway also leading to flavonols – are reduced in the *ugt78d1 ugt78d2* double mutant. Taken together, the loss of flavonol 3-*O*-glycosylation leads to a reduced flavonol biosynthesis, which may be mediated by the down-regulation of PAL activity.

P563 Regulation of ABA signalling and lipid catabolism by the N-end rule pathway of targeted protein degradation

Tara Holman1,2, Peter D Jones1, Laurel Russell3, Anne Medhurst1, Susanna Úbeda-Tomás1, Prabhavathi Talloji4, Steven Footitt2,5, Andreas Bachmair4, Frederica Theodoulou2, Michael Holdsworth1

1University of Nottingham, Nottingham, UK, 2Rothamsted Research, Harpenden, UK, 3University of Bristol, Bristol, UK, 4University of Vienna, Vienna, Austria, 5Warwick-HRI, Warwick, UK

The N-end rule pathway is a subset of the ubiquitin-dependent proteolytic system which targets protein degradation through the identity of the amino-terminal residue of specific protein substrates. Two components of this pathway in Arabidopsis, the ubiquitin ligase, PROTEOLYSIS6 (PRT6) and arginyl-tRNA:protein arginyltransferase (ATE) were shown to regulate seed after-ripening, seedling sugar sensitivity, seedling lipid breakdown and ABA sensitivity of germination. Sensitivity of *prt6* mutant seeds to ABA inhibition of endosperm rupture reduced with after-ripening time, suggesting that seeds display a previously undescribed window of sensitivity to ABA. *prt6* alleles and the *ate1 ate2* double mutant exhibited reduced root growth which was rescued by exogenous sucrose, and the breakdown of lipid bodies and seed-derived triacylglycerol was impaired in mutant seedlings, implicating the N-end rule pathway in control of seed oil mobilisation. Possible targets of the N-end rule pathway in Arabidopsis will be discussed.

P564 Identifying downstream regulators of cytokinin signalling during cambial development in Arabidopsis

Annakaisa Elo1, Kaisa Nieminen1, Juha Immanen1, Ji-Young Lee2, Ykä Helariutta1

1Department of Biological and Environmental Sciences/Institute of Biotechnology, POB 65, FI-00014 University of Helsinki, Finland, 2Boyce Thompson Institute for Plant Research, Ithaca, NY, USA

Our knowledge of the regulation of vascular development, such as an establishment of procambial cell lines and the following differentiation into xylem or phloem has rapidly been expanding but still several major questions remain open. Even though cambial activity is instrumental for the plant secondary growth, the molecular control of the stem cell maintenance or the cell proliferation in cambium is largely unknown. We and others have been able to show that cytokinin signalling induces cambial growth and cytokinins are major hormonal regulators required for cambial development (Nieminen *et al* 2008, Matsumoto-Kitano *et al* 2008). To identify genes involved in the cambium development and activity and to identify the components downstream of cytokinin signalling, we carried out a FACS based high throughput gene expression profiling experiment. The experiment was carried out using a procambium/cambium expressed cell line marker *ARR15::GFP*. In this experiment we extracted RNA from the cambial cells representing three different developmental zones from either non-treated and cytokinin treated Arabidopsis roots. Based on our microarray profiling we could identify approximately 500 procambium/cambium enriched gene expressions, from which 100 genes seem to be regulated by cytokinin. Currently we are analysing the identified genes functionally.

Matsumoto-Kitano M, Kusumotoa T, Tarkowskib P, Kinoshita-Tsujimuraa K, Václavíková K, Miyawakia K, Kakimotoa T 2008: Cytokinins are central regulators of cambial activity. Proc Natl Acad Sci U S A. 105 (50) 20027–20031.

Nieminen K, Immanen J, Laxell M, Kauppinen L, Tarkowski P, Dolezal K, Tähtiharju S, Elo A, Decourteix M, Ljung K, Bhalerao R, Keinonen K, Albert VA, Helariutta Y. 2008: Cytokinin signaling regulates cambial development in poplar. Proc Natl Acad Sci U S A. 2008 Dec 16; 105(50): 20032-7.

P565 Tissue identity shapes growth response to brassinosteroids

Yael Hacham1, Neta Holland1, Joanne Chory2, Sigal Savaldi-Goldstein1

1Technion, Haifa, Israel, 2The Salk Institute, California, USA

Organ growth depends on coordination between cell proliferation and postmitotic cell expansion. The fact that the phytohormone signaling pathways are key regulators of growth is well established but little is known about how their activity is interpreted by distinct cell types. To address this question we are using Arabidopsis roots as a system to perform local perturbation of growth via manipulation of brassinosteroids (BRs) activity. BRs primarily regulate cell expansion. However, we have found that

BRs also control the number of cells present in the elongation zone of the root meristem. Interestingly, both cell number and cell expansion are differentially regulated by distinct tissues, thus exposing various levels of cell-cell communication occurrence. Supported by recent works, our results demonstrate that BRs, like other hormones, are acting in selected cells of the plant body to regulate organ growth and serve as a basis for investigating local BR interaction with additional hormone signaling pathways.

P566 Protein tyrosine phosphorylation is involved in ABA signaling in *Arabidopsis thaliana*

Thanos Ghelis1, Gérard Bolbach2, Gilles Clodic1, Yvette Habricot2, Bruno Sotta2, Régis Maldiney2, Emile Miginiac1, Sandrine Lanfranchi1, Emmanuelle Jeannette1

1UPMC Universite Paris06, Paris, France, 2UPMC Universite Paris 06 CNRS, Paris, France

Protein tyrosine phosphorylation plays a central role in many signaling pathways leading to cell growth, differentiation and oncogenesis in animals. In higher plants, it remained controversial for a long time because a typical protein tyrosine kinase (PTK) had not been cloned. Tyrosine phosphorylated proteins have been detected in plants, the existence of protein tyrosine phosphatases (PTPs) and PTKs has been established using bioinformatics screens and their role in some physiological responses have been shown.

We investigated the role of tyrosine phosphorylation in abscisic acid (ABA) signaling using a pharmacological approach. In Arabidopsis suspension cells, phenylarsine oxide, a PTP-specific inhibitor, or inhibitors of PTKs like genistein, tyrphostin A23 and erbstatin, abolished *RAB18* gene expression induced by ABA. Stomatal closure is also blocked by phenylarsine oxide or genistein when guard cells are co-incubated with both ABA and each inhibitor. PTKs and PTPs are necessary for the ABA transduction pathways leading to *RAB18* gene expression and to stomatal closure. Genetic studies have confirmed that a PTP, PHS1, is involved in ABA signaling. A proteomic approach was conducted in order to identify the substrates of the PTKs and PTPs implicated in ABA signaling. We studied the changes in the tyrosine phosphorylation levels of proteins in Arabidopsis seeds after ABA treatment. Proteins were separated by two-dimensional gel electrophoresis and those phosphorylated on tyrosine residues were detected using an anti-phosphotyrosine antibody by Western blot. Changes were detected in the tyrosine phosphorylation levels of 19 proteins after ABA treatment. The 19 proteins were analysed by matrix-assisted laser desorption-ionization time-of-flight mass spectrometry. Among the proteins identified were three putative signaling proteins: a peptidyl-prolyl isomerase, an RNA-binding protein and a Small Ubiquitin-like MOdifier (SUMO) conjugating enzyme, these results begin to explain how tyrosine phosphorylation might regulate ABA transduction pathways in plants.

P567 RTE3, a SAC3/GANP domain protein promotes etiolated seedling growth in Arabidopsis

Christopher A McClellan, Mandy D Kendrick, Chun-Hai Dong, Caren Chang

University of Maryland, College Park, MD, USA

The *rte3 (reversion to ethylene sensitivity3)* mutant was isolated in a genetic screen for suppressors of an ethylene-insensitive mutant in etiolated seedlings. After testing other ethylene responses, such as leaf senescence, and performing epistasis analysis with other ethylene signaling mutants, it was determined that *RTE3* is unlikely to play a direct role in the ethylene signaling pathway. Instead, *RTE3* appears to be responsible for promoting hypocotyl elongation in etiolated seedlings in the ethylene triple response assay. The *rte3* mutant has *shorter* hypocotyls when grown in the dark. This phenotype is specific to etiolated seedlings, as the *rte3* mutant displays normal hypocotyl elongation when grown under white, red, blue, and far-red light sources.

The *RTE3* gene was identified by positional cloning, and is predicted to encode a protein with a SAC3/GANP domain. SAC3/GANP domains are present in proteins that participate in large multi-peptide complexes, such as the 26S proteasome regulatory subunit and the eIF3 translation initiation complex. Similarities in protein composition between these two complexes and the COP9 signalosome (CSN) suggest that a SAC3/GANP domain-containing protein may interact with members of the CSN. Interestingly, yeast two-hybrid analysis reveals that RTE3 interacts with EER5 and EIN2, proteins that have been shown to interact with members of the CSN. The *eer5* and *rte3* mutants show a similar phenotype in the ethylene triple response assay. Also of interest, *ein2-1 rte3* double mutants have a seedling growth defect that neither of the single mutants display, in which newly germinated seedlings fail to elongate. An RTE3-GFP fusion protein localizes to nuclei in root cells of four-day old seedlings when grown in white light, but this localization is abrogated when seedlings are grown in the dark. Our current hypothesis is that EIN2, EER5, RTE3, and members of the CSN form a complex to regulate Arabidopsis seedling growth. Current work on *RTE3* focuses on further phenotypic characterization and confirmation of molecular interactions.

P568 Target gene analysis of the stress tolerance gene *AtMYB44* in Arabidopsis

Jae Sung Shim, Choon Kyun Cheong, Han Yong Lee, Jong-Joo Cheong, Yang Do Choi

Department of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea

AtMYB44 belongs to the R2R3 MYB transcription factor subgroup 22 family in *Arabidopsis thaliana*. *AtMYB44* is rapidly induced by various phytohormones (abscisic acid, jasmonic acid, ethylene, etc.) and abiotic stresses (dehydration, low temperature, and salinity). Transgenic Arabidopsis overexpressing *AtMYB44* shows a rapid ABA-induced stomatal closure. This phenomenon was related to reduced expression of PP2Cs encoding genes,

which have been reported as negative regulator of ABA signaling. To identify target genes for AtMYB44, we investigated *cis*-elements for AtMYB44 by random oligo electrophoretic mobility shift assay. Using this conserved sequence, we initially screened AtMYB44 target gene candidates from promoter sequence of the whole Arabidopsis genome. Further filtration processes with salt treated microarray data produced 31 candidate genes. RT-PCR and northern blot analysis of these candidate genes showed 16 genes to be AtMYB44 responsive. Among these 16 genes, 8 loss of function mutants were obtained. Currently, we continue several lines of experiments to identify the target genes important for regulation of stress genes by AtMYB44 transcription factor during the tolerance responses upon environment stresses.

P569 A promoter region responsible for the tissue specific and jasmonate responsive gene expression of Brassica *NTR1* gene encoding a jasmonic acid carboxyl methyltransferase

Jun Sung Seo, Han Young Lee, Song Yion Yeu, Jong-Joo Cheong, Yang Do Choi

Department of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea

The *BcNTR1* gene encodes an S-adenosyl-L-methionine:jasmonic acid carboxyl methyltransferase (JMT), and is specifically expressed in the floral nectaries of *Brassica campestris* L. ssp. pekinensis. We have investigated the regulatory mechanism for the activation of *BcNTR1* gene during floral development. Series of 5'-deletion and recombination constructs of *BcNTR1* promoter were prepared and fused in translational frame to the b-glucuronidase (GUS) reporter gene. Histochemical GUS staining and Northern blot analysis revealed that the promoter region spanning about –3.8 kb to –3.1 kb from the translation initiation site is essential for the significant jasmonate responsive and tissue specific gene expression. Comparing the Arabidopsis *AtJMT* promoter to the *Brassica BcNTR1* promoter, we found a region of highly homologous sequences. Yeast-one-hybrid screening using these sequences as a bait was attempted to screen regulators for promoter activation. Three candidates were tested for the binding activity in yeast of each protein expressed from full length cDNA clone on the promoter region. Detailed experiments to identify the transcription elements responsible for the regulation of JMT activity during the floral development is under progress.

P570 Sphavata, a JA-induced AP2/ERF transcription factor of *Arabidopsis thaliana*

Beatrice Giuntoli1, Francesco Licausi2, Parlanti Sandro2, Wolfgang Dröge-Laser3, Christoph Weiste3, Pierdomenico Perata2

1Universite di Pisa, Pisa, Italy, 2Scuola Superiore Sant'Anna, Pisa, Italy, 3Albrecht-von-Haller-Institut, Universitat Gottingen, Germany

In the screening of an *Arabidopsis thaliana* ERF (Ethylene-Responsive Factor) over-expression library, the constitutive OE-ERF16 genotype, called Sphavata, was selected because of its altered leaf morphology. When grown in soil under unstressed conditions, Sphavata plants display a phenotype with reduced rosette leaves size and number, lack of petiole and delayed floral transition. ERF16 encodes a member of the A-5 subfamily of AP2/ERF transcription factor family, whose function has not been investigated so far. The gene is predominantly expressed in wild-type Col-0 roots and flower stems, while its basal level of expression in leaves is low. ERF16 appears to be responsive to wounding stress and treatment with exogenous MeJA. On the basis of the available evidence, it is possible to hypothesize a role for Sphavata in JA-related processes during plant organ development, such as bolting, leaf morphology and root growth, and, possibly, response to herbivore or pathogen attack. Arabidopsis mutants involved in the perception and transduction of the JA signal will be used to position Sphavata in the JA-dependent pathways. Moreover, overexpressor and knock-out lines will be compared for the expression of downstream JA-responsive genes.

P571 ATHB12, a homeodomain-leucine zipper (HD-Zip) class I, negatively regulates inflorescence stem length

Yoon-Sun Hur, Jong-Yoon Park, Sunghan Kim, Choong-Ill Cheon

Department of Biological Science, Sookmyung Women's University, Seoul, Korea

Homeobox genes are transcription factors that regulate numerous developmental processes including embryo development. *ATHB12* belongs to the HD-Zip class I of plant homeobox genes, and was shown to be induced by drought, cold, salt, and ABA treatments, as well as by biotic stresses such as an inoculation with *Pseudomonas syringae.* Previous studies revealed that *ATHB12* was mainly expressed in stem, petiole and leaves in response to ABA. *In situ* hybridization suggested that the expression of *ATHB12* was detected in the vascular region of stem and root. Induction by ABA or salt was tested using transgenic tobacco plants containing serial 5' deletions of the *ATHB12* promoter fused to the glucuronidase (GUS) gene. The fluorometric assay and real-time quantitative PCR revealed that the longest promoter (2.1-kb) showed the highest GUS activity and expression. These data indicate that the promoter region between 2.1-kb and 1.5-kb contains an essential element(s) for ABA-induction of *ATHB12*. We also isolated a T-DNA insertion mutant of *ATHB12* and found that T-DNA inserted -293 base pairs upstream of the transcription start site. The mutant had lower expression of *ATHB12* in the stems and showed longer inflorescence stems compared with wild type. The T-DNA insertion mutant also had a higher germination rate on ABA-containing media. The phenotype of this mutant is the reversal of that of the *ATHB12* overexpressor showing the retardation of stem elongation. Moreover, ABA treatment induced *ATHB12* expression in inflorescence stem and inhibited the stem growth, similarly in the *ATHB12* overexpressor. Our data suggest that *ATHB12*, which is induced by ABA and water deficit, might have a negative role in the regulation of stem growth in response to stress.

P572 Auxin and CUC2 pathways interact to regulate serration development

Gemma Bilsborough, Michalis Barkoulas, Miltos Tsiantis

The University of Oxford

There is remarkable diversity in leaf shape amongst plant species, including at the margins, which can be described as smooth, serrated or lobed. However, the developmental pathways that regulate margin morphology remain poorly understood. The model plant species *Arabidopsis thaliana* produces leaves with serrated margins, and two main pathways have been described as regulating serration formation.

Firstly, the auxin efflux carrier PINFORMED1 (PIN1) generates localised auxin activity foci which are required for serration formation. Secondly, the microRNA *miR164* targets *CUC2* transcripts for degradation to determine the depth of serrations.

Here we show that the two pathways interact as auxin controls the expression of *CUC2* via promotion of *MIR164A* transcription, and, in turn, *CUC2* feeds back to regulate PIN1 levels.

P573 Analysis of Arabidopsis GABA-shunt mutants

Anke Hueser, Ul Fluegge, Frank Ludewig

University of Cologne, Cologne, Germany

Gamma-amino butyric acid (GABA), a four-carbon non-protein amino acid, is the main inhibitory neurotransmitter in mammalia. It is found in all species and has a signaling function in organisms ranging from mammalia to bacteria. In plants, however, GABA accumulates under various stress conditions, but a function remains unclear. GABA is synthesized in the cytosol of plants by decarboxylation from glutamate, catalyzed by members of the small family of Glutamate Decarboxylase (*GAD*) genes. The GABA-catabolism is located in the plant mitochondria. The first step is the GABA-Transaminase (*GABA-T*) reaction, yielding succinic semialdehyde (SSA). Following oxidation by the SSA-Dehydrogenase (*SSADH*), this metabolite enters the citric acid cycle as succinate.

Plants with a non-functional *GABA-T* display normal vegetative growth but upon transition to the generative phase, these mutants reveal a reduced fertility compared to wild type plants. A mutation in the *SSADH* gene leads to plants that are retarded in growth and development and develop necrotic lesions under standard greenhouse conditions. When comparing growth characteristics of GABA-shunt mutants to wild type plants *in vitro*, *ssadh* mutant plants were found to develop undifferentiated tissue at the shoot-root transition zone.

The same effect could be induced in Arabidopsis wild type plants by growing them on media supplemented with SSA, where the rate of callus formation was found to be concentration dependent.

P574 Auxin selectivity in the AtTIR1 family

Sarah Lee1, Lynne Armitage2, Martin Kieffer2, Paul Drayton3, Richard Napier1, Stefan Kepinski2

1Warwick HRI, University of Warwick, Wellesbourne, CV35 9EF UK, 2Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK, 3Syngenta, Jealott's Hill International Research Centre, Braknell, Berkshire, RG42 6EY, UK

The hormone auxin plays a central role in plant growth and development, by regulating gene transcription and protein expression. This mechanism involves the F-box protein TIR1, and a small family of related auxin-regulated F-box proteins (the AFBs) which act as receptors for these responses.

The AFBs have both unique and overlapping contributions to auxin perception and it is likely that there is also selectivity for their ubiquitination substrates. Indeed, evidence suggests that each key component of the TIR1/AFB signalling complex contributes to biological selectivity within the auxin signalling system.

We are expressing members of the TIR1 family in order to describe the kinetics of interaction between receptor and hormone, quantify auxin selectivity between TIR1 family members and the selectivity of TIR1 for its Aux/IAA substrates. We show baculovirus-mediated expression of TIR1 and AFB5 and their auxin-dependent interactions with Aux/IAA degron peptides using surface plasmon resonance measurements from a Biacore 2000.

Comparisons of auxin-selectivity for TIR1 and AFB5 suggest quite different affinities for IAA.

Bioenergy

P575 New insight into regulatory mechanisms of TOC159 in the *Arabidospis thaliana* chloroplast protein import machinery

Birgit Agne, Charles Andres, Bastien Christ, Cyril Montandon, Felix Kessler

Institute of Biology, University of Neuchatel, Neuchatel, Switzerland

Chloroplast protein import and biogenesis are essential for plant energy and biomass production.

The Toc- (translocon at the outer membrane of the chloroplast) and Tic- (translocon at the outer membrane of the chloroplast) complexes constitute the quantitatively most important preprotein import pathway into the chloroplast.

The Toc core complex consists of two membrane bound and surface exposed preprotein receptor GTPases, Toc159 and Toc33 associating with a protein translocating channel, Toc75.

Here, we will report on the hyper-phosphorylation of domains of Toc159 and on the identification of kinases involved.

P576 F8H functions redundantly with FRA8 for glucuronoxylan biosynthesis in Arabidopsis

Chanhui Lee, Zheng-Hua Ye

Department of Plant Biology, University of Georgia, Athens, Georgia, USA

Xylan is a predominant cell wall polymer primarily found in the secondary cell wall (SCW) of woody plants. Arabidopsis has been used as a model for studying cell wall biosynthesis during wood formation because of the presence of thick SCW in interfascicular fiber and the functional conservation of cell wall biosynthetic gene orthologs between Arabidopsis and Poplar. Recently, several research groups have identified several glycosyltrasnferase (GTs) involved in xylan biosynthesis. Here, we demonstrate that FH8, a close homolog of FRAGILE FIBER 8 (FRA8), is involved in glucuronoxylan biosynthesis and functions redundantly with FRA8. The *F8H* gene is preferentially expressed in xylem cells, and the F8H protein is targeted to the Golgi, in which GX biosynthesis occurs. Overexpression of *F8H* in the *fra8* mutant completely restored the secondary wall thickness of fibers and vessels, GX content, and the abundance of the reducing end sequence of GX to the wild-type level. The plants homozygous for *f8h* did not exhibit any detectable phenotypes including SCW thickness and stem strength. However, the *f8h/fra8* double mutant resulted in an additional reduction in cell wall xylose level, a more severe deformation of vessels, and an extreme retardation in plant growth compared with the *fra8* mutant. Taken together, our results indicate that F8H performs the same biochemical function as FRA8 and they function redundantly in GX biosynthesis during secondary wall formation in the xylem.

P577 Plastoglobules: Chloroplast lipid droplets in plant stress responses

Céline Besagni Andrès, Lucia Eugeni Piller, Shanmugabalaji Venkatasalam, Jacopo Martinis, Nadège Fahrni, Felix Kessler

Institute of Biology, University of Neuchatel, rue Emile Argand 11, CH-2007 Neuchatel, Switzerland

Plastoglobules are chloroplast lipid droplets. They are often attached to the thylakoid membrane and therefore believed to act in thylakoid lipid metabolism. Under stress conditions (e.g senescence, nitrate starvation, oxidative damage) resulting in chlorophyll and thylakoid membrane breakdown, plastoglobules enlarge due to the accumulation of metabolic products. New results on plastoglobules ultrastructure, proteins and lipids composition, suggest their active participation in various metabolic pathways. As an example, VTE1 (tocopherol cyclase) associates with plastoglobules. Most likely the cyclase reaction takes place at the lipid droplets surfaces contributing to the accumulation of tocopherol (Vitamin E).

Besides VTE1, plastoglobules contain several other proteins with predicted enzymatic functions including: lipases, NAD(P)H dehydrogenase or carotenoid dioxygenase. Through the functional characterisation of these proteins, we will discover by which mechanisms plastoglobules are involved in plants stress responses.

For most of the new plastoglobules proteins, we have tools such as T-DNA insertions lines and transgenic lines expressing different recombinant proteins (GFP, YFP, CFP, 6-Histidine tags or Tandem Affinity Purification (TAP)). Here, we will present systematic techniques we use on plants to observe chloroplasts ultrastructure and plastoglobules organisation (size, number, density, proteome).

P578 Identification and characterization of genes and gene products involved in secondary cell wall biosynthesis and deposition

Julian Verdonk1, David Rancour1, Christine Ondzighi1, Cynthia Cass2, Yury Bukhman1, John Sedbrook2, Marisa Otegui1, Patrick Masson1, Sebastian Bednarek1

1Great Lakes Bioenergy Research Center, University of Wisconsin-Madison, Madison, WI, USA, 2Great Lakes Bioenergy Research Center, Illinois State University, Normal, IL, USA

Secondary walls of vascular cells account for the majority of carbohydrates in plant biomass and are synthesized following primary cell wall formation during tracheary element (TE) differentiation. We are using *in vitro* TE differentiation of Arabidopsis suspension cultured cells as a model system for understanding secondary cell wall biosynthesis and deposition. Arabidopsis T87 cells can be differentiated into both meta- and protoxylem TEs in up to 75% of the culture population through brassinosteroid application. The differentiating T87 cell cultures are being used for proteomic and biochemical analyses of the secondary cell wall formation. Principle component analysis of existing datasets of gene expression during TE formation has yielded novel genes whose expression correlates with the differentiation of TEs. We are currently testing the role of candidate gene function in secondary cell wall biosynthesis through (1) *in planta* reverse genetics in Arabidopsis, and (2) genetic manipulation and expression in transformed Arabidopsis T87 cell lines. Generation and transformation of constructs expressing candidate genes (e.g. encoding cell wall biosynthesis enzymes or intracellular transporters) is underway. Biochemical analyses of differentiating TE cell wall composition from wild-type and transgenic T87 cells, coupled with *in planta* loss-of-function allele analysis, will be used to assess the functional role of candidate gene function during secondary cell wall biosynthesis. Improved understanding of secondary cell wall deposition will provide a means for the production of crops with enhanced biomass yield that can be applied to biofuel production.

P579 Exploiting natural variation in *Arabidopsis thaliana* to understand cell wall biosynthesis and composition

Katy M Christiansen, A Michelle Smith, Dominique Loque, Joshua L Heazlewood

Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Emeryville, CA, USA

Genetic variation in different accessions of *Arabidopsis thaliana* has occurred through thousands of years of

geographic spread and adaptation. As a result of this diversity, disparate accessions have phenotypic differences that can be used to identify genes that contribute to the biosynthesis and composition of cell walls. Forward and reverse genetic screens to identify single mutants often prove difficult for detection of genetic differences that may lead to more subtle phenotypes. Exploiting the inherent genetic variation in Arabidopsis accessions through quantitative trait analysis will allow for the detection of variation in cell wall biosynthesis and composition. We have resequenced two Arabidopsis accessions, Bay-0 and Shahdara, in collaboration with the Joint Genome Institute.

This effort has resulted in a collection of SNPs between these accessions and the reference Arabidopsis accession, Col-0. Additionally, six more accessions are in the process of being resequenced. QTL analysis is beginning with determining parental phenotypes for monosaccharide composition, lignin content, and cell wall structural information. Through the use of RILs for the parental accessions, we hope to identify genes that contribute to composition and biosynthesis to further our understanding of plant cell walls.

P580 Genomics based analysis of cell wall signalling and metabolic processes in *Arabidopsis thaliana*

Lucinda Denness1, Alex Wormit1, Priya Madhou1, Lars Kjaer1, Christopher Somerville2, Thorsten Hamann1

1Imperial College, London, England, 2Energy Biosciences Institute, Berkeley, CA, USA

Lignocellulosic derived biofuels are a possible alternative to fossil fuels. Current projections suggest biofuels derived from second generation bioenergy crops will have superior energy efficiency, decreased production costs and lower carbon emissions compared to first generation biofuels and fossil fuels. Cellulose, the principle plant cell wall polysaccharide, is a major glucose source for bioethanol production. Current research suggests that plants are capable of modulating cell wall structure and composition. Therefore, characterising genes involved in cell wall remodelling could provide tools capable of modifying cell walls to optimise industrial processing. To identify genes involved in cell wall remodelling cell wall stress was generated by treating Arabidopsis seedlings with an inhibitor of cellulose biosynthesis (isoxaben, CBI) and a time course expression profiling experiment performed. Phenotypic characterisation and expression analysis after 8h of CBI, detected lignin deposition and increased expression of genes involved in neutral cell wall sugar biosynthesis. Genes exhibiting transcriptional changes in this timeframe and significant expression in the root elongation zone, stem or hypocotyls of Arabidopsis (e.g. plant organs undergoing cell wall formation or remodelling), were considered candidates for cell remodelling processes. Homozygote T-DNA insertion lines have been identified for the candidate genes and all confirmed knock out lines phenotypically characterised via phloroglucinol staining (to detect lignin deposition), and Fourier Transform InfraRed (FTIR) clustering analysis. Genes showing FTIR or lignin phenotypes are being further analysed (linkage analysis via diagnostic enzyme digestion, neutral cell wall sugar measurement, cellulose and uronic acids measurement). Candidate genes identified as a result of this research, will form the means to optimise bioenergy crops, either through accelerated molecular marker assisted breeding programs or GM based approaches.

P581 Isolation of Golgi apparatus sub-compartments from *Arabidopsis thaliana* cell suspension cultures

Harriet Parsons, Jun Ito, Katie Christiansen, Joshua L Heazlewood

Joint BioEnergy Institute, Lawrence Berkeley National Laboratories, Berkeley (California) USA

Synthesis of hemicellulose and pectin is believed to be located in the Golgi apparatus. The plant Golgi is a dynamic, fragmented organelle; different polymer backbones and sidechains may be synthesised in particular Golgi sub-compartments. Techniques such as isotope-tagging have increased the number of known Golgi-localised proteins. However, contamination by other organelles has limited the characterisation of the plant Golgi apparatus so that much of the information regarding the total number of enzymes involved in hemicellulose synthesis, their compartmentalisation and biochemistry is missing.

This project aims to isolate intact Golgi and post-Golgi structures from Arabidopsis cell suspension cultures for proteomic analysis, using density centrifugation and Free Flow Electrophoresis (FFE). FFE has been successfully used in the preparation of highly pure mitochondrial fractions from Arabidopsis cell suspension cultures (Eubel *et al* 2007) and in the separation of mammalian Golgi sub-compartments (Morre and Mollenhaur 2009).

In this study the distribution of organelles during density centrifugation and FFE has been followed by MS, western blot analysis and TEM. Initial results suggest that the Golgi can be fractionated by FFE into sub-compartment populations that migrate differently relative to other organelles. ER, vacuolar and plasma membranes appear to be the most common source of contamination prior to FFE. Investigations into chemical and enzymatic treatments that can enhance organellar separation during density centrifugation and FFE are ongoing. TEM images have shown intact cisternae in Golgi-enriched fractions prior to FFE. Understanding cell wall synthesis is integral to improving the yield of energy from plant biomass. Proteomics analysis of Golgi sub-compartments will provide important and novel information regarding hemicellulose biosynthesis and its regulation.

P582 Sugar homeostasis and cell wall integrity signalling pathways

Mathilde Seguela-Arnaud, Yunhai Li, Fiona Corke, Michael Bevan

John Innes Centre, Norwich, UK

Sugars, as a resource of energy and structural components, regulate many important cellular processes. In

photosynthetic and sessile organisms like plants, maintenance of sugar homeostasis requires complex regulatory mechanisms. In recent years, a pivotal role for sugars as signalling molecules has become apparent and numerous studies have been undertaken to study the molecular mechanisms of sugar regulation. Recently, isolation and characterisation of the *high sugar response mutant 8 (hsr8)* revealed a link between sugar sensing and cell wall integrity pathways. The *hsr8* mutant was isolated because it displayed, in response to sugar levels, increased dark development, increased sugar-regulated gene expression, increased starch and anthocyanin levels and reduced chlorophyll content (Li *et al,* 2007). The *hsr8* mutation was mapped to the gene encoding the first enzyme of the arabinose biosynthetic pathway.

This suggests that the defects in the cell wall composition are sensed, transduced to the nucleus, and lead to altered glucose-responsive growth and development. Genetic analysis demonstrated that the *Pleiotropic Regulatory Locus 1* (*PRL1*) was one component of this cell wall integrity pathway. The aim of our work is to use a combination of genetic and biochemical approaches to identify components of the cell wall integrity pathway and to establish the relationships between sugar-responsive and growth control pathways. The first strategy consist of a genetic screen to isolate suppressors of the *hsr8* mutation and the second strategy aims to further investigate the role of PRL1 and its putative partners in the cell wall integrity pathway. Identification of mutants and PRL1 partners will provide insights in to how sugar and cell wall integrity pathways are linked.

Peroxisome

P583 Import of metabolites into plant peroxisomes: From Arabidopsis to Adrenoleukodystrophy

Frederica Theodoulou1, Xuebin Zhang1, Carine De Marcos Lousa2, Yvonne Nyathi2, Daniela Dietrich3, Heike Schmuths3, Joceyln Baldwin2, Michael Holdsworth3, Stephen A Baldwin2, Alison Baker2

1Rothamsted Research, Harpenden, UK, 2University of Leeds, Leeds, UK, 3University of Nottingham, Nottingham, UK

Import of substrates for peroxisomal metabolism is mediated by COMATOSE (CTS; AtABCD1), an Arabidopsis homologue of the human ABC transporter, Adrenoleukodystrophy protein (ALDP). Analysis of null mutants has demonstrated that CTS plays key roles in several developmental and physiological processes, including germination, seedling establishment, fertility and root growth. The functions of CTS *in planta* can be related to the ability to metabolise distinct substrates such as fatty acids and hormone precursors via beta-oxidation. One interpretation of this finding is that CTS is a broad specificity transporter; recent progress towards testing this hypothesis by heterologous expression and purification of CTS will be presented.

Using an extensive allelic series, we have shown that the different physiological and biochemical functions of CTS can be separated by mutagenesis, thus identifying amino acid residues potentially involved in substrate specificity and/or inter-domain communication (Dietrich *et al*, 2009 MBC 20, 530). Mutant phenotypes were interpreted in the context of a homology model based on the Sav1866 crystal structure. We also demonstrated that all *cts* mis-sense mutants studied produced stable, correctly-targeted protein, in stark contrast to *ALDP*, where the majority of mutant alleles result in protein instability (http://www.x-ald.nl). This makes CTS a potentially useful model to probe the structure/function relations of ABCD proteins. We therefore examined targeting and function of human peroxisomal ABC transporters in plant cells, including wild-type ALDP and disease-associated mutants. Results from these experiments and cross-kingdom complementation approaches will be reported.

P584 Using genetics and proteomics to understand peroxisome proliferation and function

Jianping Hu

MSU-DOE Plant Research Laboratory, Michigan State Univ, USA, and Plant Biology Dept, Michigan State Univ, USA

Plant peroxisomes participate in a variety of metabolic functions, many of which are fulfilled cooperatively by peroxisomes and other subcellular compartments. The morphology, size, abundance, and positioning of peroxisomes are also highly regulated. To understand peroxisome proliferation at the molecular level, we used forward and reverse genetics to identify components of the peroxisome proliferation machinery in Arabidopsis. Besides DRP3A and DRP3B, two homologous proteins that play dual roles in the division of peroxisomes and mitochondria, another dynamin-related protein, DRP5B (ARC5), is involved in the division of peroxisomes and chloroplasts. A C-terminal tail-anchored protein, PMR1, controls the morphology and distribution of both peroxisomes and mitochondria.

These results together demonstrate that peroxisomes, mitochondria, and chloroplast share some common components in their division apparatus, indicating that coordinated division and positioning may occur between these metabolically linked organelles. To discover novel peroxisomal proteins, especially those with low abundance, we performed an in-depth proteome analysis of Arabidopsis leaf peroxisomes and subsequently tested the subcellular targeting of YFP fusions for selected candidate proteins. Peroxisomal localization was verified for 19 novel proteins that either contain predicted peroxisome targeting signals type 1 or 2 (PTS1/2) or PTS-related peptides, or lack conventional targeting signals; two new PTS1s and a new PTS2 were established.

Our study identified new proteins that potentially perform novel metabolic and regulatory functions of peroxisomes, thus exemplifying an important step toward mapping the complete plant peroxisomal proteome.

P585 Genome-wide analysis of the clofibrate-responsive transcriptome in *Arabidopsis thaliana*

MariCruz Castillo, Alberto Coego, Jose Leon

Instituto de Biologia Molecular y Celular de Plantas (CSIC-UPV), Valencia, Spain

Peroxisomes are subcellular organelles involved in processes like photorespiration, fatty acid beta-oxidation and detoxification of active oxygen species. The different functions exerted by peroxisomes are based on the extraordinary plastic changes in enzyme content and alterations in number and localization during development and also in response to the environment. The number of peroxisomes per cell is mostly controlled by proliferation processes that are modulated by endogenous and exogenous signals. As previously reported in animals, the proliferation of peroxisomes in plants can be triggered by chemicals such as clofibrate (CFB). However, in contrast to the well known function of CFB as an artificial ligand of Peroxisome Proliferator Activated Receptors in mammals, the mode of action of CFB as peroxisome proliferator and regulators of gene expression in plants remains unknown. A genome-wide analysis of the Arabidopsis transcriptome allowed the identification of 474 CFB-responsive genes. Around 50 % of them were up-regulated by CFB. A Gene Ontology analysis pointed to a significant overrepresentation of CFB up-regulated genes coding for cytochrome P450s, UDP-glucosyltransferases and glutathione transferases, proteins involved in phases I and II of typical detoxification responses. On the other hand, most of the CFB down-regulated genes coded for either defence-related proteins or enzymes of carbohydrate, lipid or nucleic acid metabolism. CFB seems to be perceived by Arabidopsis plants mainly as a xenobiotic, inducing a general detoxification response that may require the proliferation of peroxisomes.

A large-scale screening of T-DNA lines, carrying insertions in a large representation of loci of the Arabidopsis genome, is being carried out to identify genes involved in perception/signaling of CFB. We have identified a T-DNA mutant displaying partial insensitivity to CFB. The corresponding mutated gene is being functionally analyzed.

P586 Plant peroxisomal detoxification reactions mediated by glutathione-dependent enzymes

Amr Kataya, Sigrun Reumann

Centre for Organelle Research, Stavanger, Norway

Plant peroxisomes play essential roles in the detoxification of reactive oxygen species (ROS). The ascorbate-glutathione cycle has been characterized biochemically in pea peroxisomes about 10 years ago, but two enzymes, peroxisomal glutathione reductase (GR) and dehydroascorbate reductase (DHAR), have not been cloned from any plant species. We identified candidates for both proteins in our proteome analyses of Arabidopsis leaf peroxisomes (Reumann *et al*, 2007; Reumann *et al*, 2009). GR carries a yet unknown but PTS1-related tripeptide, TNL>, while DHAR even lacks any recognizable PTS. Peroxisome targeting of both proteins was confirmed *in vivo* for the full-length proteins, and TNL> was characterized as a novel PTS1 tripeptide. The enzymes are heterologously expressed in *E. coli* to determine their physiological activities. An analytical system is being developed to measure the flux capacity of the entire ascorbate-glutathione cycle in isolated leaf peroxisomes. Arabidopsis T-DNA insertion lines are being isolated and phenotypically characterized to further investigate the physiological function of the metabolic pathway under stress conditions of high hydrogen peroxide production and catalase inactivation. Because the active site of another member of the peroxisomal ascorbate-glutathione cycle, ascorbate peroxidase (APX3), faces the cytosolic side, we will further investigate which cycle intermediates pass the peroxisomal membrane.

Additionally we identified five glutathione-S transferases in the proteome of Arabidopsis leaf peroxisomes and were able to verify some of them as peroxisomal *in vivo*. Some enzymes, such as all three members of the GSTT subfamily, all of which are peroxisomal (Reumann *et al*, 2007; Dixon *et al*, 2009), are predicted to function *in vivo* as glutathione peroxidases that detoxify hydroperoxides. We use a combination of computational science, biochemistry, genetics, molecular and cell biological methods to further define the physiological function of the enzymes.

P587 Peroxisome-associated matrix protein degradation in Arabidopsis

Matthew Lingard, Melanie Monroe-Augustus, Bonnie Bartel

Rice University, Houston, TX, USA

Peroxisomes are ubiquitous eukaryotic organelles housing diverse enzymatic reactions, including several that produce toxic reactive oxygen species. Although understanding of the mechanisms whereby enzymes enter peroxisomes with the help of peroxin (PEX) proteins is increasing, mechanisms by which damaged or obsolete peroxisomal proteins are degraded are not understood. We have exploited unique aspects of plant development to characterize peroxisome-associated protein degradation (PexAD) in Arabidopsis. Oilseed seedlings undergo a developmentally regulated remodeling of peroxisomal matrix protein composition in which the glyoxylate cycle enzymes isocitrate lyase (ICL) and malate synthase (MLS) are replaced by photorespiration enzymes. We found that mutations expected to increase or decrease peroxisomal H_2O_2 levels accelerated or delayed ICL and MLS disappearance, respectively, suggesting that oxidative damage promotes peroxisomal protein degradation. ICL, MLS, and the beta-oxidation enzyme thiolase were stabilized in the *pex4-1 pex22-1* double mutant, which is defective in a peroxisome-associated ubiquitin-conjugating enzyme and its membrane tether. Moreover, the stabilized ICL, thiolase, and an ICL-GFP reporter remained peroxisome associated in *pex4-1 pex22-1*. ICL also was stabilized and peroxisome associated in *pex6-1*, a mutant defective in a peroxisome-tethered ATPase. ICL and thiolase were mislocalized to the cytosol but only ICL was stabilized in *pex5-10*, a mutant

defective in a matrix protein import receptor, suggesting that peroxisome entry is necessary for degradation of certain matrix proteins. Together, our data reveal new roles for PEX4, PEX5, PEX6, and PEX22 in PexAD of damaged or obsolete matrix proteins in addition to their canonical roles in peroxisome biogenesis.

P588 Peroxisomal targeting of PEX7, a receptor for PTS2-containing proteins, to peroxisomes

Makoto Hayashi, Tanuja Singh, Shoji Mano, Yuko Arai, Shino Goto, Mikio Nishimura

Department of Cell Biology, National Institute for Basic Biology, Okazaki 444-8585, Japan

The peroxisome is an organelle that accomplishes a variety of physiological functions such as lipid catabolism and photorespiration. Most of the peroxisomal enzymes involved in these processes contain one of two peroxisomal targeting signals known as PTS1 and PTS2 within their amino acid sequence. Of these, PTS2 has been demonstrated to be recognized by a soluble protein import receptor, PEX7. We analyzed the molecular function and subcellular localization of PEX7. Transgenic Arabidopsis overexpressing GFP-PEX7 showed defective glyoxysomal fatty acid β-oxidation, but no significant effect on leaf peroxisomal glycolate metabolism. GFP-PEX7 localizes primarily to the peroxisome. GFP-PEX7 accumulated primarily in peroxisomal membrane fractions, while endogenous PEX7 was distributed evenly in cytosolic and peroxisomal membrane fractions. The results indicated that both endogenous PEX7 and GFP-PEX7 are competent to target to peroxisomal membranes. In contrast, the peroxisomal targeting of PEX7 to peroxisomes was disturbed in cells of *pex12*, *pex13* and *pex14* knock-down mutants. Bimolecular fluorescence complementation analysis revealed that PEX7 binds directly to PTS2-containing proteins and PEX12 in the peroxisomal membrane. Overall, our results suggest that the targeting of PEX7 to peroxisomes requires four proteins: a PTS2-containing protein, PEX12, PEX13 and PEX14.

P589 Targeting and activity of COMATOSE, an Arabidopsis peroxisomal ABC transporter

Yvonne Nyathi1 Frederica L Theodoulou2, Steve Baldwin1, Alison Baker1

1University of Leeds, Leeds, UK, 2Rothamsted Research, UK

COMATOSE (CTS) is a full length ABC transporter, localised in the peroxisomal membrane of Arabidopsis and other plants, that plays an important role in plant growth and development. CTS is homologous to PXA1 and PXA2, which transport long chain fatty acyl CoAs into peroxisomes for β-oxidation in yeast. Genetic and physiological evidence suggests that CTS mediates transport of a range of substrates, including fatty acids and hormone precursors, but there is no direct evidence for this to date.

To investigate the role of CTS in fatty acid transport, the protein was expressed in *Saccharomyces cerevisiae.* We confirmed that it was targeted and stably integrated into the peroxisomal membrane with NBDs facing the cytosol. CTS was found to bind and hydrolyse ATP, suggesting it is still functional. This is the first study to demonstrate that CTS has ATPase activity. To investigate substrate specificity, we are testing the ability of CTS to complement a yeast *Δpxa1Δpxa2* mutant for growth on oleate, and investigating whether potential substrates can stimulate basal ATPase activity.

P590 Probing the binding kinetics between PEX5 and PTS1 using fluorescence anisotropy

Nicola Stephanie Skoulding, Alison Baker, Stuart Warriner

University of Leeds, Leeds, UK

Peroxisomes contain no DNA thus all proteins required for their function are required to be imported from the cytosol into the peroxisomal matrix. Proteins destined for the peroxisome contain one of two peroxisomal targeting sequences (PTS). PTS1, a tri-peptide found on the N-terminal of proteins is targeted by the cytosolic receptor PEX5 which shuttles between the cytosol and the peroxisome. The PTS1 sequence binds to the C-terminal domain of PEX5 which consists of 7 tetratricopeptide (TPR) repeat motifs.

We have expressed and purified a full length and a truncated version of PEX5 which contains just the TPR domain. Using a fluorescence anisotropy assay with lissamine-rhodamine labelled YQSKL model peptide we could demonstrate a kd in the nM range for both proteins. This system will allow us to explore the effect of various parameters and PEX5 binding partners on the affinity for the PTS1.

P591 Identification of a novel inhibitor of β-oxidation in *Arabidopsis thaliana*

Laura-Anne Brown, Stuart Warriner, Alison Baker

University of Leeds, Leeds, UK

Peroxisomes are small metabolic organelles that are involved in a number important functions in Arabidopsis including defence against oxidatative stress, β-oxidation of long-chain fatty acids, photorespiration and auxin and jasmonate signalling.

To gain further insight in the function and biogenesis of peroxisomes we employed a chemical genetics strategy where small molecules were screened for an effect on peroxisomal protein import and morphology. One chemical isolated from the screen was seen to cause peroxisomal clustering around enlarged lipid bodies and dark-grown seedlings in the presence of the compound require exogenous sucrose for normal growth. Distribution of other organelles and the morphology of the actin cytoskeleton is not affected by the compound. Fatty acid profiling has revealed that very long chain acyl CoAs accumulate and TAGs are retained. These effects are consistent with a defect in β-oxidation and target identification is currently underway.

P592 Inside the peroxisome

B Rafeiza Khan, Robert Barlow, Bethany Zolman
University of Missouri - St. Louis, St. Louis MO USA

Peroxisomes contain hundreds of enzymes that act in numerous metabolic pathways. Fatty acid beta-oxidation is essential for early seedling development in Arabidopsis and other oilseed plants. Plant peroxisomes also are implicated in photomorphogenesis, lateral root formation, and jasmonic acid synthesis required for wounding responses. Genetic evidence suggests that indole-3-butyric acid (IBA) is converted to the active auxin indole-3-acetic acid (IAA) in a peroxisomal process similar to fatty acid beta-oxidation. Our recent work has focused on studying the molecular mechanism of IBA beta-oxidation. *ibr3* has altered responses to IBA. *IBR3* encodes a peroxisomal enzyme with aminoglycoside phosphotransferase and acyl-CoA dehydrogenase/oxidase domains, making IBR3 a candidate enzyme for acting in IBA oxidation. However, mutants defective in peroxisomal fatty acid beta-oxidation enzymes also show IBA-resistant phenotypes. Five members of the acyl-CoA oxidase (ACX) family of enzymes influence IBA responses, and studies of other phenotypes in *acx* mutants show similar redundancy. Therefore, multiple enzymes may be acting in IBA metabolism or, more likely, disruptions in individual peroxisomal pathways can affect other processes. We are carrying out biochemical and genetic tests to examine the activity and specificity of IBR3 and ACX proteins in peroxisomal processes and to determine the roles of each protein in plant growth and development.

P593 A model of peroxisomal primary metabolism in Arabidopsis

Nicholas O'Toole1, Ian Castleden1, John Bussell2, Holger Eubel2, Etienne Meyer2, Nicolas Taylor2, Wenxu Zhou3, A Harvey Millar2, Steven M Smith2,3, Ian Small2

1Centre of Excellence in Computational Systems Biology, University of Western Australia, Perth, Australia, 2ARC Centre of Excellence in Plant Energy Biology, University of Western Australia, Perth, Australia, 3Centre of Excellence in Plant Metabolomics, University of Western Australia, Perth, Australia

Energy metabolism in plants is largely conducted by mitochondria, plastids and peroxisomes. The enzymes present in these organelles are being identified from proteomics data in combination with fluorescent protein tagging and bioinformatics predictions. We are combining these data with information on enzymatic reactions from Aracyc and KEGG to construct metabolic networks for these energy organelles. These networks are a starting point for quantitative modeling of energy metabolism in plants. Initially we have generated a model of peroxisomal metabolism including all enzymatic reactions involving primary metabolites. Flux balance analysis of the model recovers experimentally determined pathways classically associated with peroxisomes and provides evidence for ignoring other less well established pathways that have been proposed to be located within the organelle. Systematic simulations of knock-outs in each enzymatic step have been used to validate the model against known metabolic phenotypes of peroxisomal mutants and predict phenotypes for mutants that have yet to be obtained or studied.

P594 Role of peroxisomal ROS in the toxicity of herbicide 2,4-D

D M Pazmiño1, M Rodríguez-Serrano1, M C Romero-Puertas1, I Sparkes2, C Hawes2, J León3, L A del Río1, L M Sandalio1

1Estacion Experimental del Zaidin, CSIC, Apartado 419, E-18080 Granada, Spain, 2Research School of Biological & Molecular Sciences, Oxford Brookes University, Oxford, OX3 0BP, UK, 3Instituto de Biologia Molecular y Celular de Plantas, CSIC-UPV, E-46022 Valencia, Spain

Peroxisomes are cell organelles with an essentially oxidative metabolism involved in different metabolic pathways. 2,4-dichlorophenoxyacetic acid (2,4-D) is an analogue compound to indole-3-acetic acid (IAA), which is used either as a growth-promoting substance or a herbicide, depending on its concentration. Reactive oxygen species (ROS) have been involved in the mode of action of this herbicide, although the source of ROS and the signal transduction pathway have not been well established.

In this work using pea and Arabidopsis plants, the effect of 2,4-D on peroxisomal ROS production was studied. The effect of ROS in oxidative damage and signalling was investigated by biochemical, cellular and transcriptomic approaches. Treatment with 2,4-D induced epinasty in young leaves which was associated with H_2O_2 accumulation, as detected by histochemistry with DAB and confocal laser microscopy. The peroxisomal enzymatic sources of H_2O_2, acyl-CoA oxidase and xanthine oxidase, were induced by the herbicide, and the overproduced H_2O_2 brought about protein oxidation. Actin was one of the oxidized proteins and this produced disturbances in the cytoskeleton and peroxisomal dynamics. The role of peroxisomal H_2O_2 in the regulation of cell response to 2,4-D was studied by transcriptomic analysis with an Arabidopsis mutant deficient in *acx1*.

Supported by the Ministry of Science and Innovation (BIO2008-04067) and Junta de Andalucía (P06-CVI-1820), Spain.

P595 Peroxisomal thiolases in metabolism, signaling and development

Andrew Wiszniewski1,2,3, John Bussell1, Steven Smith1

1ARC Centre of Excellence in Plant Energy Biology, 2The Universiy of Western Australia, 3Perth, Australia

Arabidopsis has three genes encoding type-I peroxisomal 3-ketoacyl CoA thiolase - *KAT1, KAT2* and *KAT5*. The *kat2* mutant is deficient in fatty acid beta-oxidation and seedlings have altered number, size, structure and mobility of sub-cellular organelles. Flowering of *kat2* is prolonged, as if impaired in global proliferative arrest (GPA). Thus KAT2 is implicated in multiple metabolic and signaling systems in development. KAT1 and KAT5 are of unknown function, but the *KAT5* gene is expressed coordinately with genes of flavonoid synthesis and it encodes cytosolic and peroxisomal isoforms. *kat1, kat5*

and a *kat1 kat5* double mutant have no visible or growth phenotype. However a *kat2 kat5* double mutant shows sucrose-dependent germination and seedling growth, and subsequent plant growth is so impaired that no flowers are produced. The phenotype of the *kat2 kat5* double mutant is unlike other beta-oxidation mutants, implying a novel function for KAT5 particularly at later stages of development. We are investigating if such KAT5 function is cytosolic or peroxisomal, and in which tissues it occurs. We cannot produce a *kat1 kat2* double mutant, showing that KAT5 alone cannot fulfill all thiolase functions. Thus the peroxisomal thiolase family is implicated in multiple essential and potentially novel functions which we are now dissecting genetically.

P596 The peroxisomal/glyoxysomal processing protease DEG15 in higher plants

Esther Dolze, Christine Gietl

Technische Universitat Munchen, Germany

Glyoxysomes are a subclass of peroxisomes involved in lipid mobilization. Two distinct peroxisomal targeting signals (PTSs), the C-terminal PTS1 and the N-terminal PTS2, are defined. Processing of the PTS2 for protein import is conserved in higher eukaryotes. The cleavage site typically contains a Cys at positions P1 or P2. The glyoxysomal processing protease (GPP) was purified from the fat-storing cotyledons of watermelon *(Citrullus vulgaris)*. Specific antibodies against the peroxisomal DEG-protease from Arabidopsis (DEG15) identified the watermelon GPP as a DEG15. A knockout mutation in the *DEG15* gene of Arabidopsis (At1g28320) prevents processing of the glyoxysomal malate dehydrogenase precursor to the mature form. The DEG15/GPP appears in two forms with different substrate specificities. The 72-kDa monomer is a general degrading protease and the 144-kDa dimer constitutes the processing protease. The equilibrium between these two forms is shifted upon Ca^{2+} removal toward the monomer and upon Ca^{2+} addition toward the dimer.

We tried to overexpress the *DEG15*-cDNA in *E. coli, N. tabacum* and *P.pastoris* in order to obtain the necessary protein amounts for biochemical characterization and structure analysis.

The Arabidopsis *deg15*-knockout mutant was complemented with the *DEG15*-full length-cDNA and several deletion constructs for a functional analysis of the different DEG15 domains. We will identify the function of a conserved loop-domain near the catalytic triad, which differs the plant DEG15 from all other DEG-proteases. Since DEG15 has no PDZ-domains, we will investigate the function of the N- and the C-terminus concerning their function in protein-protein interaction and substrate recognition.

Helm, M., Lück, C., Prestele, J., Hierl, G., Huesgen, P.F., Fröhlich, T., Arnold, G.J., Adamska, I., Görg, A., Lottspeich, F., and Gietl, C. (2007) Proc. Natl. Acad. Sci. USA 104, 11501-11506. Dual specificities of the glyoxysomal/peroxisomal processing protease Deg15 in higher plants.

P597 The peroxines PEX2 and PEX10 show different functions in glyoxysomal and leaf peroxisomal development

Georg Hierl, Jakob Prestele, Christine Gietl

Technische Universitat Munchen, Freising, Germany

Glyoxysomes play a central role for storage lipid mobilization during plant germination. For fast lipid body degradation glyoxysomes increase dramatically in number, and form a glyoxysomal reticulum in the cells of dark grown cotyledons. Imbibed seeds contain already a high number of glyoxysomes and a further increase is observed in subsequent days of development in the dark until day 3. Confocal and electron microscopic analyses revealed in the course of fatty acid degradation a network-like aggregation of the glyoxysomes with lipid bodies. After 5-6 days of germination, most of the lipid bodies are degraded and the number of glyoxysomes has decreased but still exceeds the number of leaf peroxisomes in green tissue. Plants expressing PEX2 with a dysfunctional Zn-Finger in WT genetic background (Pex2ΔZn) exhibit an impaired matrix protein import. Corresponding plants carrying a PEX10 with a dysfunctional Zn-Finger (Pex10ΔZn) exhibit a reduction of glyoxysomal clusters. Beside lipid mobilization photorespiration is crucial for plant vitality. By overexpressing PEX10 with a dysfunctional Zn-Finger in the WT genetic background (Pex10ΔZn) we observed loss of contact between leaf peroxisomes and chloroplasts resulting in a phenotype typical for photorespiratory mutants. PEX10 is proven to be an E3-ubiquitin ligase involved in the recycling of PEX5. We observed only slight defects in matrix protein import in Pex10ΔZn lines containing GFP-PTS1 as marker. Metabolomic analysis showed a different metabolite distribution in Pex10ΔZn as compared to WT and Pex2ΔZn. We conclude that PEX10 has more than one function in the peroxisomal membrane and is not only involved in the matrix protein import but also important for peroxisome-chloroplast contact and metabolite flow between organelles during photorespiration. To prove our hypothesis we will localize PEX10 and PEX2 within the peroxisomal membrane.

Schumann U., Prestele, J., O'Geen H., Brueggemann, R., Wanner, G., Gietl, C. (2007) Requirement of the C3HC4 zinc RING finger of the Arabidopsis PEX10 for photorespiration and leaf peroxisome contact with chloroplasts. Proc. Natl. Acad. Sci. USA 104 1069-1074

P598 Different metabolic roles for aconitase isoforms during establishment of Arabidopsis seedlings

Sarah Nicholl1, Lorrie Murphy2, Mark Hooks1

1School of Biological Sciences, Collage of Natural Sciences, Bangor University, Bangor LL57 2UW, UK, 2School of Chemistry, College of Physical and Applied Sciences, Bangor University, Bangor LL57 2UW, UK

In plants, aconitase catalyses the reversible isomerisation of citrate to isocitrate in mitochondria and the cytosol as parts of the TCA cycle and glyoxylate cycle,

respectively. There are three genes in Arabidopsis, *ACO1* (At4g35830), *ACO2* (At4g26970) and *ACO3* (At2g05710), and all are expressed in developing seedlings. However, it appears that ACO3 is the only activity induced during germination and establishment comprising up to 80% of total aconitase activity at 2 days post imbibition. Since the *aco1* and *aco2* mutants show little decrease in total aconitase activity, we expressed His-tagged ACO1 and ACO2 in *Pichia pastoris* and determined aconitase activity. In physiological tests, *aco3* showed delayed emergence, but other ACO activity was necessary for seed germination, since treatment of the *aco3* mutant with fluoroacetate inhibited germination. Citrate levels were found to be nearly six-fold higher in *aco3* than in wild-type or either *aco1* or *aco2* indicating that the mitochondrial ACO3 was primarily responsible for metabolizing citrate that would come from lipid mobilization. Interestingly, [2-14C] acetate feeding to 3-day-old mutants showed that only *aco2* deviated substantially from wild-type in the profile of radiolabel incorporation into various metabolite classes. Mutant *aco1* did not show any deviation from wild-type in either citrate levels or radiolabelling suggesting that little, if any, citrate is metabolised by the cytosolic aconitase, a step of the classic glyoxylate cycle. We conclude that mitochondrial ACO3 predominates in citrate metabolism in establishing seedlings, but the roles each play in seedling carbon metabolism remain poorly defined.

P599 *PED3* is required for process of breaking dormancy

Masatake Kanai, Mikio Nishimura, Makoto Hayashi
National Institute of Basic Biology, Aichi, Japan

Seed dormancy is an important mechanism for seed survival. Seed dormancy is broken during imbibition, and starts a sequence of programs for germination. Germination is a complicated process. Forward genetical screening revealed that several genes are involved in dormancy, but it is still unclear how dormancy is broken.

It is reported that *PED3*, a peroxisomal ABC transporter, is required for breaking dormancy and that the germination ratio of *ped3* mutant is lower than 10%. To identify genes that act as a trigger for breaking dormancy, we investigated transcriptional changes during seed imbibition in *ped3*. Microarray analysis revealed that expression of some dormancy-related genes decreases as normal, whilst other genes were increased to abnormally high levels after imbibition in *ped3*.

We showed that promoter regions of genes up-regulated in *ped3* contained ABA-responsive elements (ABREs). These results indicate that *ped3* partially maintains dormancy after imbibition and perturbs ABA signaling. We analyzed the function of genes that were expressed at abnormally high levels in *ped3* and related to ABA signaling.

Authors Index