TIMETABLE – MONDAY 24 JUNE 2013

Time	*Location*	*Event*
07:30-20:00	Parkside Foyer	**Registration**
18:00-20:00	Parkside Foyer	***Opening / Welcome Mixer / Exhibition***

TIMETABLE – TUESDAY 25 JUNE 2013

Time	*Location*	*Event*
08:30-09:15	Parkside Auditorium	**Keynote Lecture** **Dr Detlef Weigel** ***"****Arabidopsis thaliana and its relatives as model systems for the study of evolutionary questions"*
09:15-10:00	Parkside Auditorium	**Keynote Lecture** **Professor Ralph Bock** "*Experimental genome evolution in plants"*
10:00-10:30	Parkside Foyer	***Morning Tea / Exhibition / Posters***
10:30-11:00	Parkside Auditorium	**Plenary Lecture** **Professor Dominique Bergmann** "*Making a difference: asymmetry, fate and self-renewal in the stomatal lineage"*
11:00-11:30	Parkside Auditorium	**Plenary Lecture** **Professor Ian Small** "*A code for RNA recognition by pentatricopeptide repeat proteins"*
11:30-12:00	Parkside Auditorium	**Plenary Lecture** **Professor Xiao Feng Cao** "*Flowering time regulation mediated by protein arginine methylation"*

TIMETABLE – TUESDAY 25 JUNE 2013

Time	*Location*	*Event*
12:00-12:50	Parkside Foyer	***Lunch / Exhibition / Posters***
12:50-14:20	Parkside Auditorium	**Workshop 1** EPIC: Epigenomes of Plants International Consortium
12:50-14:20	Parkside 110B	**Workshop 2** Redox Signaling in Mitochondria
14:30-16:00	Parkside Auditorium	**Symposium 1** Natural Variation, Evolution and Phenomics I
14:30-16:00	Parkside 110B	**Symposium 2** Hormones
16:00-16:30	Parkside Foyer	***Afternoon Tea / Exhibition / Posters***
16:30-18:00	Parkside Auditorium	**Symposium 3** Development I
16:30-18:00	Parkside 110B	**Symposium 4** Photosynthesis and Drought
18:00-20:00	Parkside Foyer	**Mixer / Exhibition / Posters**
18:15-19:15		***Odd numbered posters to be manned by presenting authors***

TIMETABLE – WEDNESDAY 26 JUNE 2013

Time	*Location*	*Event*
08:30-09:00	Parkside Auditorium	**Plenary Lecture** **Professor Joseph Ecker** "*Regulatory networks controlling hormone-mediated growth"*
09:00-09:30	Parkside Auditorium	**Plenary Lecture** **Professor Miltos Tsiantis** "*Towards understanding development and diversity of leaf shape"*
09:30-10:00	Parkside Auditorium	**Plenary Lecture** **Professor Peter Waterhouse** "*The generation and spread of small RNAs in plants"*
10:00-10:30	Parkside Foyer	***Morning Tea / Exhibition / Posters***
10:30-12:00	Parkside Auditorium	**Symposium 5** Translational Biology
10:30-12:00	Parkside 110B	**Symposium 6** Development II
12:00-12:50	Parkside Foyer	***Lunch / Exhibition / Posters***
12:50-14:20	Parkside Auditorium	**Workshop 3** International Arabidopsis Informatics Consortium: The Transition from TAIR to the Arabidopsis Information Portal (AIP)
12:50-14:20	Parkside 110B	**Workshop 4** Living Imaging of Protein Functions

TIMETABLE – WEDNESDAY 26 JUNE 2013

Time	*Location*	*Event*
14:30-16:00	Parkside Auditorium	**Symposium 7** Small RNA, RNA and Epigenetics
14:30-16:00	Parkside 110B	**Symposium 8** Cell and Organelle Biology
16:00-16:30	Parkside Foyer	***Afternoon Tea / Exhibition / Posters***
16:30-18:00	Parkside Auditorium	**Symposium 9** Emerging Technologies and Systems Biology
16:30-18:00	Parkside 110B	**Symposium 10** Energy Biology and Metabolism
18:00-19:30	Parkside Foyer	***Happy Hour / Exhibition / Posters***
18:15-19:15		***Even numbered posters to be manned by presenting authors***

TIMETABLE – THURSDAY 27 JUNE 2013

Time	*Location*	*Event*
08:30-09:00	Parkside Auditorium	**Plenary Lecture** **Professor Julia Bailey-Serres** "*Exploring the "mRNPome": profiling stress-triggered dynamics in mRNA sequestration and translation"*
09:00-09:30	Parkside Auditorium	**Plenary Lecture** **Professor Mark Stitt** "*Balancing the carbon budget, does Arabidopsis do a better job than bankers and politicians?"*
09:30-10:00	Parkside Auditorium	**Plenary Lecture** **Professor Keiko Sugimoto** "*Developmental control of plant cell growth"*
10:00-10:30	Parkside Foyer	***Morning Tea / Exhibition / Posters***
10:30-12:05	Parkside Auditorium	**Symposium 11** Simon Chan Memorial Symposium
10:40-12:05	Parkside 110B	**Symposium 12** Cell to Cell Communication
12:05-12:50	Parkside Foyer	***Lunch / Exhibition / Posters***
12:50-14:20	Parkside Auditorium	**Workshop 5** The Small Regulatory Molecules: microRNAs and Peptides
12:50-14:20	Parkside 110B	**Workshop 6** Programme Cell Death during Arabidopsis Development and Stress Response

TIMETABLE – THURSDAY 27 JUNE 2013

Time	*Location*	*Event*
14:30-16:00	Parkside Auditorium	**Symposium 13** Transgenerational Inheritance
14:30-16:00	Parkside 110B	**Symposium 14** Abiotic Stress
16:00-16:20	Parkside Foyer	***Afternoon Tea / Exhibition / Posters***
16:05		***Passport Prize Draw***
16:20-17:05	Parkside Auditorium	**Keynote Lecture** **Professor Kazuo Shinozaki** "*Regulatory gene networks in drought stress response and tolerance"*
17:05-17:50	Parkside Auditorium	**Keynote Lecture** **Professor Joanne Chory** "*Retrograde signaling during development and high light stress"*
17:50-18:00	Parkside Auditorium	**Student & Early Career Researcher Poster Prize Awards /** **ICAR 2014 Presentation**
18:30-23:15	Italian Village Restaurant	***Cruise to Cocktail Dinner***

TIMETABLE – FRIDAY 28 JUNE 2013

Time	*Location*	*Event*
08:45-10:15	Parkside 110A	**Symposium 15** Natural Variation, Evolution and Phenomics II
08:45-10:15	Parkside 110B	**Symposium 16** Biotic Interactions
10:15-10:45	Parkside Foyer	***Morning Tea***
10:45-12:15	Parkside 110A	**Symposium 17** Proteins and Postranslational Regulation
10:45-12:15	Parkside 110B	**Symposium 18** Signaling and Gene Regulation
12:15-12:45	Parkside Foyer	***Lunch***
12:45-14:15	Parkside 110A	**Workshop 7** Genetic Traits from Phenomics Data
12:45-14:15	Parkside 110B	**Workshop 8** Teaching Workshop for Early Career Scientists
14:15-14:30	Parkside Foyer	***Afternoon Tea***
14:30-16:00	Parkside 110A	**Workshop 9** Plant Nutrition in the Face of Impending Global Resource Limitation Opportunities for Model Plant Research
14:30-16:00	Parkside 110B	**Workshop 10** Using Proteomics to Identify Receptor Complexes and Signaling Events
16:00-17:00	Parkside Foyer	***Closing / Farewell Drinks***

KEYNOTE LECTURES

KEY-TUE-01

ARABIDOPSIS THALIANA AND ITS RELATIVES AS MODEL SYSTEMS FOR THE STUDY OF EVOLUTIONARY QUESTIONS

Weigel D.
Max Planck Institute for Developmental Biology, Tuebingen, Germany.

My group is addressing fundamental questions in evolutionary biology: (i) How, and how frequently, do new genetic variants arise? (ii) Why do some variants increase in frequency? (iii) And why are some combinations of new variants incompatible with each other? In collaboration with several other labs, including those of Joy Bergelson, Joe Ecker, Richard Mott, Magnus Nordborg and Karl Schmid, and with the help of Monsanto's genomics group, we are describing whole-genome variation in natural accessions of *Arabidopsis thaliana*, under the auspices of the 1001 Genomes project (http://1001genomes.org). To better understand the patterns we observe in *A. thaliana*, we are comparing within-species variation with differences to the closest relatives, *A. lyrata*, and to variation in a closely related genus, *Capsella* (collaborations with the Nordborg, Wright and Neuffer labs). On the other end of the spectrum, we are analyzing new mutations that have arisen spontaneously under laboratory conditions or in a natural mutation accumulation experiment. The latter studies take advantage of an *A. thaliana* lineage that was apparently introduced to North America in historic times and accounts for about half of the population there. We have been able to support what we see in the extant North American population by whole-genome sequencing of herbarium samples from the 19th century. Since there has been much recent excitement about the potential contribution of heritable epigenetic variation, we are complementing our studies of genetic variation with analyses of DNA methylation differences, again over different time scales: in isogenic laboratory lines, in isogenic natural lines and across species boundaries. The ultimate goal of our top-down studies is to understand how new genetic and epigenetic variation interacts with reassortment of variants after crosses and natural selection to shape geographic patterns of genetic and epigenetic diversity. To this end, we are following natural populations during the season and over consecutive years. This work in turn is complemented by forward genetic analyses, especially of detrimental combinations of sequence variants found in separate lineages. Additional information about our work can be found on our website, http://weigelworld.org.

KEY-TUE-02

EXPERIMENTAL GENOME EVOLUTION IN PLANTS

Bock R.
Max Planck Institute of Molecular Plant Physiology, Potsdam-Golm, Germany.

Plant cells contain three genomes. The genomes of the two DNA-containing cell organelles (mitochondria and chloroplasts) originate from formerly free-living bacteria, an α-proteobacterium and a cyanobacterium, that were swallowed by a host cell in a process known as endosymbiosis. The uptake of the bacterial endosymbionts was followed by massive restructuring of both the genome of the host cell (the nuclear genome) and the genomes of the two endosymbionts (the mitochondrial and chloroplast genomes). This involved the loss of dispensable and redundant genetic information and, most importantly, the large-scale translocation of genes from the organellar genomes to the nuclear genome. At the same time, speciation processes set in leading to evolutionary diversification through reproductive isolation. However, accumulating evidence suggests that even reproductively isolated plant species can continue to exchange genetic information by horizontal DNA transfer. In my talk, I will describe experimental approaches to study genome evolution in real time. I will discuss three fundamental processes in eukaryotic genome evolution: (i) the transfer of organellar DNA to the nuclear genome and the conversion of transferred organellar DNA sequences into functional nuclear genes (endosymbiotic gene transfer), (ii) the generation of new combinations of nuclear and organellar genomes, a type of horizontal gene transfer referred to as organelle capture, (iii) the combination of nuclear genomes from different plants as a mechanism leading to the formation of new species. Using transgenic tools and developing stringent selection schemes, we have reconstructed these processes in laboratory experiments and analyzed the underlying molecular mechanisms. The implications for our understanding of genome evolution, genome integrity and speciation in plants will be discussed.

KEY-THU-03

Sponsored by CSIRO Plant Industry

REGULATORY GENE NETWORKS IN DROUGHT STRESS RESPONSE AND TOLERANCE

Shinozaki K.
RIKEN Center for Sustainable Resource Science.

Drought stress is one of severe environmental stresses that adversely affect plant growth and crop production. Recent progress of plant biology has provided useful technologies for breeding of crops that can tolerate drought stress conditions. Water use efficiency of crops can be improved by its application in breeding. For the development of these technologies, we need to understand plant responses to drought stress at molecular level since higher plants respond to drought stress through various molecular and cellular processes as well as physiological ones. Many genes with various functions are induced by environmental stresses including drought, high salinity, heat and low temperature. Many stress-inducible genes play important roles in stress tolerance. Based on the genome analysis, a lot of genes have been identified that are involved in environmental responses and tolerance not only in Arabidopsis but also in crops. Recently, many plant biologists have been trying to develop drought-resistant crops by using these stress-inducible genes in combination with their promoters by transgenic technology (see Hirayama and Shinozaki, Plant J. 61:1041, 2010). Complex regulatory systems in stress-responsive gene expression and their upstream signaling molecules have been extensively analyzed. Abscisic acid is one of the most important plant hormones in drought stress responses. In the ABA-responsive gene expression in drought stress response, the ABRE cis-acting element and bZIP transcription factors (AREB/ABF) function as major regulatory factors after the accumulation of endogenous ABA. Genes for key enzymes involved in ABA biosynthesis and metabolism, and signal transduction pathways upstream of the AREB transcription factors in drought stress response have been identified. ABA receptors and signaling pathways have been elucidated upstream of the AREB transcription factor. ATP-binding cassette (ABC) transporter is involved in ABA transport in stress response (see Umezawa et al. Plant Cell Physiol. 51: 1821, 2010). We also have identified ABA-independent regulatory pathways in drought responsive gene expression. A cis-acting element (DRE/CRT) and its binding proteins, DREB2s, are important regulatory factors in draught-stress responsive gene expression. DREB2 transcription factors regulate many stress genes with various functions including galactinol synthase and LEA proteins. We have been trying to apply our drought-inducible genes for molecular breeding of drought tolerant crops in collaboration with international crop research institutes, like IRRI, CIMMYT and CIAT. Preliminary results have been obtained on the evaluation of drought tolerance of transgenic lines with stress- inducible gene constructs (see Hirayama and Shinozaki, Plant J. 61:1041, 2010). Based on genome analysis, we have recently identified sORFs encoding small peptides that are involved in drought stress response, and analyzed their functions in regulation of stress and ABA response. The functions of the small peptides in stress response will be discussed. Recent advance in DNA sequencing technology has provides us amount of genome sequence information, which has contributed to discover useful QTL marker genes involved in environmental stress tolerance. Marker assisted breeding also provides us powerful tool to develop environmental stress tolerant crops.

KEY-THU-04

RETROGRADE SIGNALING DURING DEVELOPMENT AND HIGH LIGHT STRESS

Chory J.[1], Woodson J.D.[1], Jung H.-S.[1], Sinson A.[1], Perez-Ruiz J.[1], Priest H.[2] and Mockler T.[2]
[1]The Salk Institute for Biological Studies, La Jolla, CA 92037 USA. [2]The Donald Danforth Plant Science Center, St. Louis, MO, USA.

Chloroplast signals regulate hundreds of nuclear genes during development and in response to stress. Such communication likely involves metabolites produced within plastids, but the identities of these molecules, what regulates their production, and the signaling pathways are mostly unclear. One type of retrograde signal, a “biogenic” signal, is used by plastids as they develop into chloroplasts in young leaves. When chloroplast biogenesis is blocked in young seedlings using plastid-specific translation inhibitors or drugs that lead to photobleaching, the nucleus responds by greatly reducing the expression of hundreds of genes involved in photosynthesis or chloroplast biogenesis. Our genetic screens in Arabidopsis have identified alleles of 7 gun (genomes uncoupled) genes that are needed to communicate with the nucleus during chloroplast development. Six of these genes implicate the chloroplast tetrapyrrole biosynthetic pathway, in particular enzymes around the branch point between heme and Chl biosynthesis, as a source of retrograde signals. Loss-of-function of these GUN genes (or overexpression of GUN6) leads to misexpression of photosynthesis associated nuclear genes (PhANGs) when chloroplast development is blocked. We showed that increased flux through the Ferrochelatase I (FC1) heme branch of the chloroplast tetrapyrrole pathway increases PhANG expression. The second ferrochelatase, FC2, co-localizes with FC1, but increased FC2 activity is unable to increase PhANG expression in undeveloped plastids. These data suggest a model where heme, specifically produced by FC1, may be used as a retrograde signal to coordinate PhANG expression with chloroplast development, suggesting that heme is a positive signal that coordinates nuclear and chloroplast gene expression when the plastid is functioning. In contrast, Mg-ProtoIX accumulation may indicate that the plastid has been damaged. These results demonstrate the importance of the tetrapyrrole pathway during chloroplast development. Although not always as easy to demonstrate empirically, plastids also emit “operational” signals in response to external cues and stress. Unlike developmental signals, operational signals control hundreds of nuclear genes to limit and repair damage from reactive oxygen species generated by stresses such as excess light and drought. Studies from multiple labs have implicated the accumulation of singlet oxygen and the metabolites, MEcPP and PAP in excess light stress. We identified a set of elements enriched in promoters of genes induced during high light stress. Among these were heat shock elements. T-DNA insertion lines and overexpression studies allowed us to identify a subset of three heat shock transcription factors in the early response to excess light stress. A screen for mutants using mis-expression of the ELIP2 promoter identified another 4 regulatory genes, as well as implicated alternative splicing of hundreds of genes in the response to excess light stress in Arabidopsis.

PLENARY LECTURES

PLE-TUE-01

MAKING A DIFFERENCE: ASYMMETRY, FATE AND SELF-RENEWAL IN THE STOMATAL LINEAGE

Bergmann D.C.[1, 2], Adrian J.[1], Lau O.S.[1], Davies K.[1], Northover C.[1], Rowe M.[1], Abrash E.[1], Matos J.[1] and Ballenger C.[2]
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During development, multicellular organisms create a diverse set of specialized cell types and organize these cells into functional tissues. Often this process involves establishing self-renewing populations via asymmetric cell divisions. Once established, these populations must divide and create new differentiated cells at the appropriate rate and in the appropriate place. We use stomata (epidermal structures that regulate carbon dioxide and water exchange) as a model to understand asymmetric divisions during pattern formation in plants; stomata guard cells are created via a stereotyped set of asymmetric cell divisions whose number and orientation are dictated by the interplay of cell-type specific transcription factors and local cell-cell interactions. Work from several labs over the last decade has identified key regulators of stomatal lineage cell fates. Much of the fate specification within the stomatal lineage involves regulatory logic and molecules conserved between plants and animals. The control over asymmetric divisions and cell morphogenesis, however, involves primarily plant-specific elements. Having found footholds into the questions of cell polarity and fate, we hope to move these into a larger context—how do these key restriction points integrate information from various sources into a discrete outcome, and what are the genetic networks surrounding these nodes? Current larger-scale projects are to capture cell growth, division and targeted gene expression patterns in stomatal lineage cells as a whole leaf develops and to obtain cell-type-specific gene regulation trends. I will discuss how these approaches interface with focused projects on the targets and regulation of two transcription factors, SPEECHLESS and FAMA and the themes that emerge when cell fate and developmental flexibility in this lineage relative to other self-renewing populations in the plant.

PLE-TUE-02

A CODE FOR RNA RECOGNITION BY PENTATRICOPEPTIDE REPEAT PROTEINS

Small I.D.
ARC Centre of Excellence in Plant Energy Biology, The University of Western Australia.

The pentatricopeptide repeat (PPR) is a helical repeated motif found in a huge family of RNA binding proteins (over 450 in Arabidopsis) that are essential for correct mitochondrial and chloroplast gene expression. PPR proteins bind RNA sequence-specifically and function in RNA processing, splicing, editing and initiation of translation. We used computational methods to infer a structure for these proteins and a code for nucleotide recognition involving two amino acids in each repeat. Recoding a PPR protein to bind novel RNA sequences has validated this code. The code holds (with minor variations) for P-class PPR proteins involved in determining RNA termini and for PLS-class RNA editing factors. This breakthrough allows the prediction of PPR protein binding sites, greatly accelerating analyses of their functions. We have successfully predicted PPR editing factors for several editing sites in Arabidopsis mitochondria and chloroplasts and are extending these predictions to all flowering plants. The extraordinary evolutionary plasticity of the PPR family suggests that the PPR scaffold will be particularly amenable to redesign for new sequence specificities and functions in biotechnology.

PLE-TUE-03

FLOWERING TIME REGULATION MEDIATED BY PROTEIN ARGININE METHYLATION

Cao X.F.
State Key Laboratory of Plant Genomics and Center for Plant Gene Research, Institute of Genetics and Developmental Biology,Chinese Academy of Sciences.

Protein arginine methylation plays an essential role in regulating transcription, RNA processing, nuclear transport, DNA-damage repair and signal transduction. Arginine methylation is catalyzed by a small group of protein arginine methyltransferases (PRMTs). Type I (asymmetric di-methylation) and type II (symmetric di-methylation) arginine methyltransferases represent two most important types in animals and plants. Our previous studies showed that both Arabidopsis type II arginine methyltransferase AtPRMT5 and type I methyltransferase AtPRMT10 are involved in regulating floral transition, but *atprmt5 atprmt10* double mutants display an additive effect on flowering time and *FLC* expression, indicating that the mechanisms of regulating flowering by these two PRMTs are different. In *atprmt5*, hundreds of genes, including several RNA processing factors which have flowering time phenotype, show pre-mRNA splicing defects. Furthermore, we found that the methyltransferase activity of AtPRMT10 is essential for its role in flowering time regulation. In addition, we have identified HLP1, a novel RNA binding protein, as the protein partner and substrate of AtPRMT10. Lesions in HLP1 lead to accumulated expression of the floral repressor, *FLOWERING LOCUS C (FLC)* and cause late flowering, which mimic the *atprmt10* mutants. In addition, several key components involved in AtPRMT5-mediated function have also been identified by both genetic and proteomic approaches. The detailed mechanism will be discussed.

PLE-WED-04

Sponsored by the Faculty of Science
The University of Western Australia

REGULATORY NETWORKS CONTROLLING HORMONE-MEDIATED GROWTH

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

[1]Plant Biology Laboratory. [2]Genomic Analysis Laboratory. [3]Howard Hughes Medical Institute, The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, CA 92037, USA. [4]Department of Molecular Genetics and Banting and Best Department of Medical Research, University of Toronto, ON, Canada. [5]Division of Rheumatology and the Center for Autoimmune Genomics and Etiology (CAGE), Cininati Children's Hospital Medical Center, Cincinnati, OH 45229, USA. [6]Section of Cell and Developmental Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093, USA. [7]Cellular and Molecular Pharmacology, University of California, San Francisco, 1700 4th Street, QB3, 308D, San Francisco, CA 94158. [8]Lane Center for Computational Biology, School of Computer Science, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, PA 15213, USA.

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

PLE-WED-05

Sponsored by The Company of Biologists and the journals: Development, Journal of Cell Science, The Journal of Experimental Biology, Disease Models & Mechanisms and Biology Open

TOWARDS UNDERSTANDING DEVELOPMENT AND DIVERSITY OF LEAF SHAPE

Tsiantis M.T.
Max Planck Institute for Plant Breeding Research, 50829 Köln, Germany.

A key challenge in biology is to understand how diversity in organismal form is generated. Genetic analyses in model systems have identified key regulators that sculpt the body plans of metazoa and seed plants. However, less is known about how the action of such regulators produces particular organ shapes, or how the balance of conservation versus divergence of such form regulating pathways generated the tremendous morphological diversity of multicellular eukaryotes. One impediment to answering these questions is the relative paucity of experimental platforms where genetic tools can be utilized to unambiguously study morphogenesis and its evolution in a genome-wide, unbiased fashion. To circumvent this problem we developed the Arabidopsis thaliana relative Cardamine hirsuta into a versatile system for studying morphological evolution. We aim to understand the molecular mechanisms through which leaf morphology evolved in these species, resulting in simple, undivided leaves in A. thaliana and dissected leaves with distinct leaflets in C. hirsuta. This presentation will discuss our progress towards understanding the genetic pathways that specify dissected versus entire leaf shapes and that regulate the number, position and timing of leaflet production.

PLE-WED-06

Sponsored by School of Biological Sciences, University of Sydney

THE GENERATION AND SPREAD OF SMALL RNAS IN PLANTS

Waterhouse P.[1, 2]
[1]Schools of Biological Sciences and Molecular Bioscience, University of Sydney, NSW Australia. [2]CSIRO Plant Industry, Canberra, ACT, Australia.

Small (s)RNAs are integral to the guidance of transcriptional and post-transcriptional regulation of developmental and defence processes in plants. In some of these processes, sRNAs travel from cell to cell and long distances via the phloem, in others they move short inter-cellular distances, and in yet others the sRNAs seem to be restricted to the cells in which they are produced. Some sRNAs move and propagate in a re-iterated amplification while others appear to move without amplification. In some situations there is amplification of secondary sRNAs that spread along the body of the gene, but in others there is no such spread. The subcellular localisations of the complexes involved in the biosynthesis and actions of sRNAs are also variable and in some cases contentious or ill-defined. We have used mutagenesis screens to identify components of these process and real-time in situ techniques to examine some of the cellular and sub-cellular locations. The results of the experiments and models for the movement, spread and localisation of some of these sRNA mechanisms will be presented.

PLE-THU-07

EXPLORING THE "MRNPOME": PROFILING STRESS-TRIGGERED DYNAMICS IN MRNA SEQUESTRATION AND TRANSLATION

Bailey-Serres J.[1], Sorenson R.[1], Hummel M.[1], Juntawong P.[1, 2] and Girke T.[1]
[1]Center for Plant Cell Biology, Botany and Plant Sciences, UC Riverside, CA 92521. [2]Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, Thailand, 10900.

The ease of isolation of mRNA from plant organs has inadvertantly focused our view of gene expression to the mechanisms that regulate transcript abundance. But an array of post-transcriptional and post-translational processes control gene expression. In *Arabidopsis thaliana* modulation of mRNA translation and sequestration is a prevalent response to environmental cues. For example, in seedlings briefly deprived of oxygen fewer than 5% of the individual mRNAs decline in abundance, whereas 63% are selectively excluded from translating ribosomes (i.e., polysomes). The decreased translation of circa 90% of the total mRNA content enables cells to conserve ATP during hypoxia, which is inefficiently produced by anaerobic metabolism. The selective translation of individual mRNAs occurs in response to diverse environmental stimuli and is hormonally and developmentally regulated. With the goal of obtaining data that closely reflects active protein synthesis, we established affinity purification methods to isolate mRNAs associated with ribosomes from whole organs as well as specific cell types. This enabled evaluation of discrete populations of mRNAs and exposed the remarkable variation in mRNAs translated into protein in specific cell types of seedling roots and shoots (www.efp.ucr.edu). To refine our understanding of mRNA translational regulation during hypoxia, we have mapped ribosome position and density across gene units (Ribo-seq). This quantitative positional information of ~30 nt ribosome footprints provides new significant insights into alternative splicing, translational dynamics, and protein production. As mRNAs can display rapidly reversible sequestration or even enhanced translation during stress, we are identifying the RNA binding proteins and complexes (mRNPs) involved in these processes. Using GFP- and epitope-tagging, the subcellular location as well as mRNAs associated with several RNA binding proteins have been profiled. New perspectives on mRNA translation, sequestration and turnover are emerging from this systematic assessment of the complex and flexible plant "mRNPome". Research supported by NSF grants IOS-0750811 and IOS-1121626.

PLE-THU-08

Sponsored by Australian National University

BALANCING THE CARBON BUDGET: DOES ARABIDOPSIS DO A BETTER JOB THAN BANKERS AND POLITICIANS?

Stitt M., Flis A., Ishihara H., Lunn J., Martins M., Piques M., Pyl E.-T., Sulpice R. and Wahl V.
Max Planck Institute of Molecular Plant Physiology, Golm Germany.

The daily light-dark cycle is one of the most pervasive environmental changes with which plants have to cope. While growth in the light can be directly supported by photosynthesis, carbon must be accumulated in the light and remobilised to support metabolism and growth in the night. This provides a system to investigate how plants coordinate metabolism with growth. In many plants, the main carbon store is starch. Starch is typically accumulated in a near-linear manner in the light and degraded in a near-linear manner at night, such that almost all of the starch is used by the end of the night. Starch turnover is adjusted to changes in the growth conditions, such that if less carbon is available in a 24 h cycle, a larger proportion of the carbon is used for starch synthesis during the day, and starch is degraded more slowly at night. This is crucial for optimal plant growth in carbon-limiting conditions. I will first review how starch synthesis and degradation are regulated in the face of long- and short-term changes in the environment to guarantee a continued source of carbon for growth at night. I will then discuss recent work showing that starch degradation is under the control of the circadian clock, which paces the rate of starch degradation such that starch is almost but not totally exhausted at dawn. This is counteracted by a feedback loop, in which the sugar-signal trehalose-6-phosphate acts to restrict starch degradation and link starch mobilisation to the use of carbon for growth. I will then turn to the regulation of growth. Sophisticated regulation of starch turnover can only keep the plant out of carbon starvation if there are coordinate changes in the rate at which carbon is used for growth. We are using several approaches to monitor the rate of growth including not only measurements of expansion growth but also methods that monitor the flux of carbon to cellular components, including analysis of C balances, analysis of ribosome abundance and polysome loading, and 13C flux analyses. I will discuss recent results that illustrate how the clock and carbon supply interact to regulate the timing of growth. Finally, I will discuss emerging evidence that sugars not only regulate current use of carbon, but also regulate developmental decision that set up a future demand for carbon, like the transition from juvenility and maturity, flowering and seed set.

PLE-THU-09

Sponsored by New Phytologist

DEVELOPMENTAL CONTROL OF PLANT CELL GROWTH

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Spatial and temporal control of cell growth is central for the morphogenesis of multicellular organisms. For some cell types that undergo extensive post-mitotic cell growth, such as root and leaf hair cells, coordinating the extent of cell growth with development is vital for their specialised physiology and function. Since the final size that individual cells reach is often constant under given conditions, the duration of post-mitotic cell growth is likely to be developmentally programmed. We still know surprisingly little about how cell size is determined and how developmental signals link to this control. Previous studies suggested that the extent of cell growth is linked with an increase in ploidy by endoreduplication but how developmental signals control endocycling and cell growth is not understood in both animals and plants. We have recently shown that a trihelix transcription factor, GT2-LIKE 1 (GTL1), actively terminates ploidy-dependent cell growth and its developmentally regulated expression is one of the key determinants of cell size in Arabidopsis leaf hair cells. Through genome-wide chromatin binding studies coupled with transcriptional profiling, we also demonstrated that GTL1 directly represses the transcription of CDH1/FZR/CCS52, an activator of the anaphase-promoting complex/cyclosome (APC/C), to stop the endocycle progression and ploidy-dependent cell growth. Our findings thus uncovered a previously uncharacterised key molecular link between developmental programming and cell-size control, highlighting the central role of APC/C in post-mitotic cell growth.

SYMPOSIA

SYMPOSIUM 1 – NATURAL VARIATION, EVOLUTION AND PHENOMICS I

Sponsored by School of Biological Sciences, Faculty of Science, Monash University, Australia

SYM-01-01

THE GENOTYPE-PHENOTYPE MAP IN ARABIDOPSIS

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Understanding how genetic variation translates into phenotypic variation, and how this translation depends on the environment, is fundamental to many fields of biology. We have been attempting to tackle this problem using A. thaliana as a model for many years. Because it naturally exists as inbred lines, A. thaliana can be brought into the laboratory and grown, in replicate, under different environmental conditions. As part of the 1,001 Genomes Project, we have sequenced 200 Swedish lines, and are generating multiple phenotypes, in the field as well as in the lab. We are also generating "in-between-ome" data, such as genome-wide transcription and DNA methylation data, in order to gain insight into the mechanism whereby particular polymorphisms lead to phenotypic variation. The sequencing has revealed massive variation in genome-size, mostly due to variation in 45S rDNA clusters, and evidence for strong selective sweeps. GWAS results vary greatly between phenotypes due to difference in the underlying genetic architecture. Compared to efforts in other organisms, we find much greater effects of individual loci. At the transcriptome level, a very high proportion of genes show evidence of strong cis-regulation in GWAS, with effects peaking close to transcription start sites, while trans-effects appear to be smaller, and typically interact with the environment. In terms of DNA methylation, most of the genome is unmethylated, with methylation concentrated in repetitive parts of the genome. Methylation patterns vary between genotypes, but are largely conserved across environments, although differences in overall level are seen and appear to have a genetic basis. We are now attempting to connect all layers of data to create a genotype-phenotype map.

SYM-01-02

DECODING THE COMPLEXITY OF QUANTITATIVE NATURAL VARIATION AND RESPONSE TO THE ENVIRONMENT IN *ARABIDOPSIS THALIANA*

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Following a long history of quantitative genetics in crop plants, it is now relatively popular as well to use naturally-occuring variation contained in Arabidopsis thaliana accessions as the source of quantitative genomics approaches, designed to map QTLs and try and resolve them at the gene level. Apart from being able to exploit –in multiple genetic backgrounds– allelic variation that cannot be easily retrieved from classical mutagenesis, the (relatively few) success of the QTL studies has often been because of the use of quantitative phenotyping, as opposed to the qualitative scales often used in typical mutant screens. The objective of our work is to apply genome-wide quantitative molecular genetics to both, a very integrative and classical quantitative trait (shoot growth) and a molecular trait a priori more directly linked to the source of variation (gene expression under cis-regulation), in both cases studied in interaction with the abiotic environment (especially drought stress). We are using a combination of our unique high-troughput phenotyping robot (the Phenoscope), RNA-seq, fine-mapping, complementation approaches and association genetics to pinpoint a significant number of QTLs and eQTLs to the gene level and identify causative polymorphisms and the molecular variation controlling natural diversity. Exploiting these strategies at an unprecedented scale thanks to the Phenoscope should allow to resolve enough quantitative loci to start drawing a more general picture as to how and where in the pathways adaptation is shaping natural variation. I will present recent results obtained when trying to decipher the genetic architecture of growth response to the environment, to illustrate our strategies and research. The VAST Lab : www.inra.fr/vast.

SYM-01-03

NATURAL VARIATION OF A GENE NETWORK REGULATING TRICHOME PATTERNING

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In Arabidopsis thaliana, the regulation of five different traits, namely trichome patterning, root hair patterning, seed coat mucilage formation, anthocyanin and proanthocyanin biosynthesis, is intertwined by an evolutionary conserved gene network coding for WD40, bHLH, MYB proteins.. These traits are likely to be naturally regulated as it has been shown that there are involved in protection against pathogens, UV, or germination (Calo et al, 2006; Yan et al, 2012). We are using a whole genome association mapping approach in Arabidopsis thaliana to analyse the diversity within the underlying network and to identify new genes contributing to these traits. We developed new screening techniques to analyze trichome patterning and proanthocyanidin content which allow rapid and precise phenotyping of Arabidopsis accessions. Recently, we released "TrichEratops", a software to analyze light microscopy pictures, allowing 3D reconstruction of the leaf surface and extracting known and new trichome patterning parameters. Even with low number of ecotypes (71) the first results show interesting candidates indicating that precise and fine phenotyping allow the use of genome wide association with a small batch of accessions.

SYM-01-04

NATURAL VARIATION IN THE DEVELOPMENTAL CONSEQUENCES OF A LOSS OF CHLOROPLAST TRANSLATION IN ARABIDOPSIS THALIANA

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Interfering with chloroplast translation is typically more detrimental to growth and development in Arabidopsis than in Brassica or maize. This difference appears to reflect, in part, variation in the presence and functionality of a duplicated nuclear gene encoding a plastid-localized acetyl-CoA carboxylase (ACCase) required for fatty acid biosynthesis. In this study, we demonstrate that different accessions of Arabidopsis thaliana also vary in their ability to tolerate a loss of chloroplast translation. Two different approaches were pursed to block chloroplast translation: incorporation of spectinomycin into culture media used for seedling growth; and analysis of mutants defective in genes encoding chloroplast-localized ribosomal proteins. From an initial survey of 52 early-flowering accessions germinated on spectinomycin, 9 were chosen for further analysis: 3 tolerant accessions that produced albino rosettes; 3 sensitive accessions with at most rudimentary leaves; and 3 sensitive/ intermediate accessions associated with knockouts of EMB genes encoding chloroplast-localized ribosomal proteins. When sensitive and tolerant accessions were crossed and responses of F2 plants analyzed on spectinomycin, results were in some cases consistent with a single locus conferring tolerance. Crosses were then performed between mutants defective in chloroplast translation and representatives of tolerant and sensitive accessions. This resulted in the identification of a suppressor locus that partially rescues mutant seeds and maps to a region on chromosome 1 that includes ACC2. Additional modifiers that support further embryo development were also found. Surprisingly, qPCR experiments revealed that ACC2 is not over-expressed in seedlings of the tolerant accession examined. Whether ACC2 activation in transgenic plants completely rescues mutant embryos remains to be determined. This work highlights the importance of evaluating accession-specific differences in mutant phenotypes in Arabidopsis.

SYM-02-01

INS AND OUTS OF ARABIDOPSIS PEROXISOME BIOGENESIS

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Peroxisomes compartmentalize critical oxidative reactions, thereby protecting the cytosol from oxidative damage. Plant peroxisomes compartmentalize not only fatty acid beta-oxidation and jasmonate biosynthesis, which are needed for early seedling survival and plant defense, respectively, but also the beta-oxidation of an auxin precursor to an active auxin form. This finding motivated our study of the function, biogenesis, and dynamics this vital organelle. We have employed a variety of forward-genetic screens to isolate Arabidopsis mutants defective in the biogenesis of peroxisomes. Our analyses of these mutants have revealed new matrix protein import components, unanticipated interdependencies among peroxisome biogenesis factors, and a novel pathway for degrading peroxisome matrix proteins during developmentally controlled organelle remodeling. We have uncovered several intriguing examples in which Arabidopsis peroxisomes more closely resemble mammalian peroxisomes than do yeast or nematode peroxisomes, suggesting that these studies may provide unique insights into human peroxisome biogenesis disorders.

SYM-02-02

STRIGOLACTONE BIOSYNTHESIS AND ROLES IN PLANT DEVELOPMENT

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Strigolactones are now accepted as a plant hormone with diverse signaling roles in plant development. They affect shoot branching, secondary growth, senescence, adventitious rooting and, to varying extents, lateral rooting, root hair development. They also play a substantial role in signalling nutrient deprivation from root to shoot, enabling developmental responses to nutrient deprivation, and play key roles in establishing particular nutrient related symbioses. Strigolactones are produced in shoot and root and are carotenoid-derived, with the several biosynthetic steps occurring in plastids including the production of carlactone. Biosynthetic steps after carlactone are predicted but not yet elucidated. Using a microarray approach in Arabidopsis which took advantage of auxin and feedback regulation of strigolactone biosynthesis, we identified a new gene we named LATERAL BRANCHING OXIDOREDUCTASE. LBO encodes an oxidoreductase-like enzyme of the 2-oxoglutarate and Fe(II)-dependent dioxygenase super-family and is required for branching repression. This protein which functions in shoot and root is required downstream of previously reported strigolactone biosynthesis enzymes in the conversion of carlactone to mobile bioactive strigolactone(s). LBO will be useful to identify strigolactones involved in receptor binding and signaling as well as in understanding strigolactone diversity and function.

SYM-02-03

AUXIN SIGNALING AND GROWTH RHYTHMICITY AT THE SHOOT APICAL MERISTEM

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The shoot apical meristem (SAM) generates all the aerial parts of the plant that arise after germination. Lateral organs initiated at the SAM emerge in a very precise spatio-temporal pattern called phyllotaxis. Auxin is a key signal in controlling SAM development, as its accumulation at the meristem periphery is sufficient to trigger organogenesis. We developed a new auxin signaling sensor, DII-VENUS, which consists in a fusion of the domain II of IAA28 to a fast-maturating YFP targeted to the nucleus, expressed under control of 35S promoter. By its design, DII-VENUS can monitor in vivo, with a cellular resolution, Aux/IAAs local degradation that directly depends on auxin levels. We used this tool to investigate auxin dynamics in growing SAMs. We found that auxin levels in the SAM fluctuate with a surprising regularity (with a ~24 hours period) and independently of auxin polar transport, as auxin fluctuations are also detected in pinoid and pin-formed1 mutants. We also observed that auxin fluctuations are maintained under different light conditions, suggesting that this process is controlled by an endogenous clock. Interestingly, we showed that these rhythmic hormonal pulses correlate with growth fluctuations in the SAM, which are partially abolished in the abp1+/- heterozygous line. In current phyllotaxis models, new organs are formed as the previous ones move away from the shoot tip. Thus, growth patterns should represent a key parameter in controlling timing of organ initiation at the SAM. Indeed, we provided evidence that organ initiations in the meristem do not occur linearly but mainly when growth rates are highest. Our data suggest a dual role of auxin at the SAM, both in cell identity and in global growth regulation (through the ABP1 pathway) that, in turn, determines timing of organ initiation.

SYM-02-04

DAD2, A PROTEIN INVOLVED IN STRIGOLACTONE PERCEPTION

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Strigolactones are a class of plant hormones involved in branching, leaf senescence, root development, and plant-microbe interactions. They are carotenoid-derived lactones, synthesized in the roots and transported acropetally to modulate axillary bud outgrowth (i.e. branching). We have identified the DAD2 gene from petunia, an orthologue of the rice and Arabidopsis D14 genes, and show evidence for its role in strigolactone signal transduction. The crystal structure of DAD2 reveals an α/β hydrolase fold containing a canonical catalytic triad with a large internal cavity capable of accommodating strigolactones. DAD2 can interact with the F-box protein PhMAX2A in a strigolactone dependent manner at nanomolar concentrations. DAD2 is destabilised in the presence of strigolactone, potentially indicating conformational change in the presence of the hormone. These observations suggest that DAD2 acts to bind the mobile strigolactone signal and then interacts with PhMAX2A to initiate an SCF-mediated signal transduction pathway. DAD2 also has slow enzymatic activity, hydrolysing its ligand. Mutation of the catalytic triad abolishes catalytic activity and the ability of DAD2 to interact with PhMAX2A. The hydrolysis products of DAD2 can neither stimulate the protein-protein interaction nor modulate branching, suggesting that DAD2 could be the strigolactone receptor.

SYM-03-01

THE DEVELOPMENTAL PHASE TRANSITION TO FLOWER FORMATION

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Onset of flower formation triggers a transition from biomass and resource production in the leaves and branches to allocation of these resources to the next generation in the flowers. Timing of this switch and of subsequent flower development are thus important for both plant survival and for agriculture. In the monocarpic plant Arabidopsis thaliana, onset of reproduction occurs in two steps. First, the floral transition causes cessation of rosette leaf formation and upwards growth of the shoot apical meristem (bolting). Next, the meristem identity transition triggers formation of the reproductive structures, the flowers. My lab is interested in elucidating the inputs that control the optimal timing of the meristem identity transition. In addition, we are studying the regulation of early events in flower morphogenesis. Much of our work has focused on the plant specific helix-turn-helix transcription factor LEAFY (LFY), which plays a critical role in both processes. When LFY is induced, it directs newly formed primordia in which it is expressed to adopt a floral fate. Recently, we have identified endogenous regulators of LFY expression that include a microRNA-dependent age-sensing pathway and the phytohormone auxin. In addition, we have elucidated key events set in motion by LFY that lead to formation of a flower meristem. I will report on our progress in understanding of this vital developmental phase transition.

SYM-03-02

USING BRYOPHYTE MODELS TO UNDERSTAND LAND PLANT EVOLUTION

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The origin of land plants (embryophytes) was one of the most important evolutionary events in the earth's history. The spread and diversification of a land flora changed the biosphere and made possible the subsequent colonization of land by metazoans, allowing the origin of complex terrestrial ecosystems. Molecular phylogenetic and ultrastructural data indicate that land plants are most closely-related to the charophycean algal group Charales and were thus likely derived from freshwater, aquatic ancestors from which they inherited numerous developmental, biochemical, and cell biological features. However, the origin and diversification of embryophytes involved dramatic evolutionary changes in life history, physiology, and body plan that allowed for more complex forms adapted to life on land. Key features associated with land plant evolution were the origin of a multicellular diploid sporophyte (from the retained zygote) and a resulting alternation of generations, a gametophytic apical meristem with an apical cell that divides in multiple planes producing 3-dimensional tissues, a sporophytic shoot apical meristem (SAM) with a capacity for branching, lignified vascular tissues, production of lateral organs (leaves) from the SAM, and the origin of roots, and stomata to regulate gas exchange and water loss; and many other morphological and physiological adaptations to life on land. Liverworts, hornworts, and mosses, commonly referred to as bryophytes, are the earliest-diverging extant lineages of land plants. Based on the fossil record, liverworts are the basal most lineage and some extant species, such as Marchantia polymorpha, may retain at least some ancestral features of land plants. We have been establishing Marchantia as a model genetic system to investigate both the evolution of body plans and genome evolution in land plants.

SYM-03-03

THE BOUNDARY-SPECIFIC TRANSCRIPTION FACTOR LATERAL ORGAN BOUNDARIES LIMITS GROWTH BY REPRESSING BRASSINOSTEROID ACCUMULATION

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Leaves and flowers begin life as outgrowths from the periphery of the shoot apical meristem. Organ formation initiates when a population of founder cells on the periphery of the shoot apical meristem (SAM) is specified to form an organ primordium. Founder cells undergo transcriptional reprogramming and altered rates and patterns of cell division to produce the initiating organ; meanwhile stem cell divisions in the SAM replenish cells lost to organogenesis. Organ boundaries isolate the meristem from the initiating organ and are critical for coordination of organogenesis and meristem maintenance. The Arabidopsis boundary specific transcription factor LATERAL ORGAN BOUNDARIES (LOB) functions to limit growth of boundary cells, allowing organ separation. LOB expression is regulated by brassinosteroids (BRs) and ectopic LOB activity results in reduced BR responses. Microarray experiments identified the BR-inactivating enzyme BAS1 as a target of LOB transcriptional regulation, suggesting that LOB is a negative regulator of BR accumulation. Furthermore, we show that hyper-accumulation of BRs in the organ boundary results in fusion defects similar to those of the lob mutant. Our data indicate the presence of a feedback loop involving BR regulation of LOB accumulation and LOB repression of BR accumulation. The work demonstrates the developmental importance of regulating BR responses and indicates that formation of a BR minimum in organ boundaries is critical for boundary integrity.

SYM-03-04

THE C2-DOMAIN PROTEIN QUIRKY AND THE ATYPICAL RECEPTOR-LIKE KINASE STRUBBELIG LOCALIZE TO PLASMODESMATA AND MEDIATE TISSUE MORPHOGENESIS IN *ARABIDOPSIS THALIANA*

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

SYM-04-01

REGULATION OF PHOTOSYNTHETIC ELECTRON TRANSPORT BY PSI CYCLIC ELECTRON TRANSPORT

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Light reactions of photosynthesis consist of linear and photosystm I (PSI) cyclic electron transport. Whereas linear electron transport generates both ATP and NADPH, PSI cyclic electron transport preferentially contributes to ATP synthesis. In angiosperms, PSI cyclic electron transport consists of the main pathway depending on PGR5-PGRL1 proteins and the chloroplast NDH complex. The main pathway is essential for dissipating excessive absorbed light energy from PSII (qE induction) and also ATP supply for CO2 fixation. Although the contribution of NDH is minor under the growth chamber conditions, it is important under the pgr5 mutant background, suggesting that chloroplast NDH is required for alleviating oxidative stress in chloroplasts. In Arabidopsis, NDH forms the supercomplex with PSI to stabilize NDH especially at high light intensity. This has happened in the evolutional process of land plants. I discuss the central role of PSI cyclic electron transport in the redox homeostasis in photosynthesis.

SYM-04-02

RETROGRADE SIGNALLING AND DROUGHT

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Plant organelles produce retrograde signals that can alter nuclear gene expression in response to developmental or environmental cues. Signals of different chemical nature have been proposed in the past decades, including chlorophyll intermediates, reactive oxygen species, and oxidation products of carotenoids. It is expected that some of the signals be important during abiotic stress conditions, such as drought and excess light, to maintain homeostasis or optimize cellular performance. The levels of the phosphonucleotide 3'-polyadenosine 5'-phosphate (PAP) are regulated by the dual localized (chloroplast and mitochondria) phosphatase SAL1. PAP concentration increases during drought and after high light stress in Arabidopsis. Using a transgenic approach, we found evidence that PAP can move between the organelle and the nucleus complementing molecular and morphological phenotypes. PAP is also an inhibitor of yeast 5' to 3' exoribonucleases (XRNs), and it could regulate plant gene expression by altering RNA metabolism mediated by XRNs because SAL1 and nuclear XRN mutants share similar morphological phenotypes, have improved drought tolerance and more than 50% of altered genes are co-regulated. Extensive genetic and physiological evidence shows that SAL1, and by inference PAP, regulates several developmental and physiological processes in Arabidopsis. I will present new evidence linking this retrograde signalling pathway with ABA signal transduction during regulation of stomatal aperture.

SYM-04-03

LOCALIZATION AND MEMBRANE INTERACTION OF THE ZEAXANTHIN EPOXIDASE

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The xanthophyll zeaxanthin (Zx) has a central photoprotective function in the short- and long-term response of plants to high light stress. Zx is not only essential for the pH-regulated energy dissipation in the time range from seconds to minutes, but is also supposed to be involved in the down-regulation of photosystem II at longer time scales (up to several hours). In addition to that, Zx is known to contribute to photoprotection independent of its role in energy dissipation. The Zx epoxidase (ZEP) activity and hence the reconversion of Zx to violaxanthin was found to be gradually down-regulated with increasing light stress. These characteristics imply that Zx may act in both the short-term and long-term as a molecular memory of light stress in plants, although the molecular basis of these properties is not understood. It is unknown whether this unique function may be related to the fact that Zx serves not only as photoprotective xanthophyll in the thylakoid membrane but also as precursor of the stress hormone abscisic acid (ABA). To date, the exact localization within the chloroplast and the molecular basis of the light-dependent regulation of the ZEP are largely unknown. Using specific antibodies raised against the ZEP of Arabidopsis thaliana, we have analyzed the distribution of the ZEP protein across different plant tissues. Furthermore, we studied the localization of the ZEP within the chloroplast and its interaction with the thylakoid membrane in dependence of different light treatments. Our data provide new insights into the localization and light regulation of the ZEP in relation to its function in energy dissipation and ABA biosynthesis.

SYM-04-04

CHLOROPLAST GENE EXPRESSION IN C_4 *CLEOME*

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The C_4 cycle enhances photosynthesis by concentrating CO_2 from mesophyll (M) cells into RuBisCO-containing bundle sheath (BS) cells. Cell-specific accumulation of proteins, especially in the chloroplast, is critical for an efficient C_4 cycle. Although the mechanisms governing cell-specific expression of nuclear genes have been extensively investigated, little is known about the mechanisms that give rise to M- and BS-specific expression of chloroplast genes, despite the primordial role of chloroplast-encoded RuBisCO in C_4 photosynthesis. To complete our understanding of the processes that generate cell-specific accumulation of proteins used in the C_4 pathway, we have analysed transcript abundances across the whole chloroplast genome in both M and BS cells in Cleome, the genus containing the closest known C_4 relatives of Arabidopsis. Chloroplast transcripts show up to 35-fold differences in leaves of the closely related species C. gynandra (C_4) and C. spinosa (C_3). However, these differences are mostly not due to M- or BS-specific accumulation of chloroplast transcripts. The similarity of the M and BS chloroplast transcriptomes in C. gynandra contrasts with the striking differences at the protein level and allows us to rule out transcriptional regulation and RNA degradation as major determinants of cell specific expression, as they would lead to observable differences in steady-state RNA levels. Most likely, cell-specific chloroplast gene expression in C. gynandra is regulated at the translational or post-translational level.

SYMPOSIUM 5 – TRANSLATIONAL BIOLOGY

Sponsored by ARC Centre of Excellence in Plant Cell Walls

SYM-05-01

PLANT CELL WALLS: FROM CELL BIOLOGY TO BIOENERGY AND HUMAN HEALTH

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The central roles of plant cell walls as renewable sources of transport fuels and as functional foods that improve human health are now widely recognised. Cellulose, lignin and other wall polysaccharides represent the world's largest renewable carbon resource. As a major source of dietary fibre cell walls are also important determinants of human health. There has been steady progress towards identifying the genes that mediate the biosynthesis of major wall components, but associated regulatory mechanisms involved in the synthesis, deposition, re-modelling and depolymerisation of these wall components remain undefined. Our experimental approaches have been focused on the grasses, although we routinely use Arabidopsis as a model system for proof-of-function of genes of interest. Genetic information on the biosynthesis, remodelling and degradation of the most important wall polysaccharides in the grasses, namely the heteroxylans, (1,3;1,4)-beta-glucans and cellulose, will be presented in the context of the biological functions of individual wall polysaccharides. In addition to their role in wall structure and in the modification of wall properties during normal growth and development, and in response to abiotic and biotic stresses, constituent polysaccharides might also be important as a secondary store of metabolizable sugars in the plant. Carbon partitioning between starch, wall polysaccharides and oils is attracting renewed interest in the field. In addition, generally accepted views of the mechanisms of wall polysaccharide assembly in the endomembrane system of cells are being re-evaluated. Examples of how advances in our fundamental knowledge of plant cell wall biology can be translated into the areas of renewable biofuel production and improved human health will be presented.

SYM-05-02

ABSTRACT NOT AVAILABLE

SYM-05-03

THE USE OF ARABIDOPSIS FOR CEREAL GRAIN DORMANCY STUDIES

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Seeds from dicot and monocot plants exhibit a number of striking differences in their anatomy and responses to the environment. Arabidopsis seeds are characterised by a relatively large embryo surrounded by the residual endosperm (single cell aleurone layer). In contrast cereals grains are characterized by a very large endosperm (starchy endosperm and aleurone layer) and a relatively small embryo to one side of the caryopsis. Recent work from our lab and others has shown that there appears to be strong conservation of dormancy and germination mechanisms at the gene and physiological level between Arabidopsis and cereals, but also notable differences between the species. We have used the knowledge gained from model plants like Arabidopsis to functionally examine components and pathways that regulate dormancy in wheat and barley. Our data support the key role of abscisic acid in controlling seed dormancy in both Arabidopsis and cereals and we have found an example of convergent evolution of developmentally different tissues in imposing dormancy in cereals and Arabidopsis. We have also compared the effect of light quality on germination and dormancy of Arabidopsis, wheat, barley and the wild model grass, Brachypodium. We found that Brachypodium germination not only exhibited red/far-red responses similar to that found in Arabidopsis but also blue light responses that appear to be restricted only to grasses. We have proposed that the red/far-red regulation of germination in wheat and barley may have been lost during domestication. Finally, we have successfully used Arabidopsis to functionally test the role of barley and wheat dormancy-related genes.

SYM-05-04

THE USE OF ARABIDOPSIS AS RESOURCE TO IMPROVE FIELD PENNYCRESS, A NEXT GENERATION BIODIESEL FEEDSTOCK

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In much of the Midwestern United States, large portions of the landscape do not have a living cover during the fall and winter months following the harvest of corn or soybean. The lack of a living plant cover leaves soil vulnerable to erosion and nutrient runoff. Field pennycress (Thlaspi arvense L.) has been shown to be an effective winter cover crop and biodiesel feedstock that can be planted following the harvest of soybean and harvested in the spring in time for planting the next crop of soybean. The integration of pennycress into the current farming system provides a beneficial winter cover and a new source of biofuel without displacing land for food production. To date, there has been extremely limited breeding to improve the agronomic characteristics of pennycress. Pennycress is closely related to Arabidopsis, thus the translation of the basic knowledge gleaned from Arabidopsis should stimulate rapid improvement in pennycress. The power of next-generation sequencing is being used to develop genomic tools needed for the development of improved pennycress germplasm. Traditionally, the short-read sequencing from the Illumina platform has proven computationally difficult, but we have configured and built a personal computer for less than $2000 US able to perform transcriptome and genome assembly using the software CLC Genomics Workbench. We have sequenced, assembled, and annotated the transcriptome of pennycress, and the genome sequencing and assembly is currently in progress. We will provide an update on the status of these activities and present a detailed comparative transcriptome analysis between the Arabidopsis and a winter annual variety of pennycress.

SYMPOSIUM 6 – DEVELOPMENT II

Sponsored by EMBL Australia

SYM-06-01

THE ESTABLISHMENT OF LATERAL ORGAN POLARITY IN ARABIDOPSIS

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Organ formation is a fundamental developmental process in plants and animals. It involves a positioning mechanism that defines where the tissue or organ will arise as well as changes to growth and differentiation that result in morphogenesis and the correct patterning of cell types. By confocal imaging of multiple GFP-labeled proteins we have begun to gain an overall picture of how these interrelated processes are coordinated. The picture that emerges is one of cross-regulation at multiple levels. For instance, we find that central-peripheral cell-type patterning in the shoot localizes auxin response such that initiating lateral organs are able to inherit their adaxial/abaxial cell type patterning directly from meristem precursor tissue. This is in part mediated by auxin-responsive cell polarity patterns that not only help to further localize auxin via its polar transport, but also regulate morphogenesis at the cellular level via the microtubule cytoskeleton. In turn, localized auxin within the periphery feeds back to cell type patterning which results in changes to the domains of auxin response and future organ development.

SYM-06-02

SEARCHING UPSTREAM REGULATORS OF VERNALIZATION INSENSITIVE3, AN EARLY INITIATOR OF VERNALIZATION RESPONSE

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Vernalization, a prolonged winter cold to accelerate flowering, confers plants competence to flower at spring. Most of the Arabidopsis accessions collected from northern areas show strong response to vernalization due to the presence of FLOWERING LOCUS C (FLC), a strong floral repressor, of which expression is highly suppressed by vernalization. VERNALIZATION INSENSITIVE 3 (VIN3) encoding a PHD finger domain protein is induced by vernalization and initiates a series of epigenetic suppression of FLC. So far, we do not know how plants sense vernalization signals and distinguish it from the short-term cold. VIN3 may provide the clue to this question because the expression is induced not by short-term cold but by vernalization. To get some insight into this question, we dissected the VIN3 promoter and searched upstream regulators of VIN3 by mutagenesis using VIN3p-GUS as parental line. We found that VIN3 promoter has multiple regulatory elements, both positive and negative, and narrowed down the promoter into 200 bp for the vernalization response. We will also discuss our recent results regarding upstream regulators of VIN3.

SYM-06-03

SPRINGING INTO FLOWER AFTER WINTER: CONTROL OF FLOWERING IN MEDICAGO

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The timing of flowering is an important regulator of crop productivity and plant adaptation. There are commonalities in flowering time control such as FT-like genes which encode a mobile protein florigen that promotes flowering. However, there is also much diversity. Different plants respond to different signals differently and some encode unique regulators. In plants from temperate climates, flowering time is often regulated by seasonal signals such as photoperiod (day length) and extended exposure to cold (vernalisation). Flowering time regulation in these plants is best understood in Arabidopsis and the cereals barley and rice. Less is known about the control of flowering in other important groups of plants such as the Legumes. Therefore, we are currently aiming to expand knowledge of the genetic control of flowering in eudicots, and ultimately our ability to better customize flowering in crops, by investigating flowering control in a model temperate legume Medicago truncatula (Medicago). Medicago has a number of attractive advantages for use as a model plant including transposon-tagged mutant lines, diploid genetics and a sequenced genome. We are carrying out forward genetic screens of mutant populations and doing reverse genetics with candidate flowering-time genes. We have identified spring mutants that flower early independently of vernalisation. Results include identification of an interesting allelic set of dominant retroelement-insertion mutants with insertions in or downstream of a Medicago FT gene. In this talk, flowering pathways from different plants will be compared and our progress on molecular identification and analysis of spring mutants presented.

SYM-06-04

AUXIN CONTROLS GRAVITROPIC SETPOINT ANGLE IN HIGHER PLANT LATERAL ORGANS

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A fundamental feature of plant architecture is that many organs, including the primary root and shoot and lateral branches are maintained at specific angles with respect to gravity, a quantity known as the gravitropic setpoint angle (GSA). While the GSA of the primary axis is typically approximately vertical, the GSA values of lateral shoots and roots are most often non-vertical, allowing the plant to optimise the capture of resources both above- and below-ground. This spatial regulation of GSA is manifest throughout nature in the form of characteristic species-specific patterns of GSA control, perhaps most conspicuously in the diverse branching patterns of trees. The mechanisms by which GSA is set and maintained are not known. Here we show that the non-vertical GSAs of lateral shoots and roots depend upon an angle offset that is the product of latent balancing gravitropic and anti-gravitropic asymmetries in auxin response. We show that this anti-gravitropic offset, which is supressed in the primary shoot, can also be switched off in the sub-apical shoot branch following removal of the shoot apical meristem, a transition that is also regulated by auxin. Finally we show that auxin specifies GSA values dynamically throughout development by regulating the magnitude of the anti-gravitropic offset and further, that variation in auxin sensitivity in a single cell type is sufficient to alter lateral branch GSA. The involvement of auxin in controlling GSA is yet another example of auxin's remarkable capacity to self-organise in development and provides a conceptual framework for understanding the specification of GSA throughout nature.

SYMPOSIUM 7 – SMALL RNA, RNA AND EPIGENETICS

Sponsored by Molecular Plant

SYM-07-01

THE BIOGENESIS AND FUNCTIONAL DIVERSITY OF PLANT SMALL RNAS

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Non-coding RNAs, especially small RNAs, play important roles in many biological processes. Plant small RNA types including microRNAs (miRNAs) and small interfering RNAs (siRNAs), as well as secondary siRNAs that include trans-acting siRNAs (tasiRNAs). Many small RNAs along with their targets have been characterized with deep sequencing technologies, but much remains to be learned about their function. My lab has developed a suite of computational tools for the analysis and visualization of data for small RNAs and cleaved mRNAs (miRNA targets). We have applied these tools to a range of plant species and their variants, such as mutants in small RNA biogenesis or downstream pathways. This has led to the characterization of a number of new miRNAs and their targets, as well as the identification of diverse populations of phased, secondary siRNAs and their miRNA triggers. We also have applied these methods to the analysis of plant mutants in small RNA stability and turnover, including the methyltransferase HEN1, an enzyme which stabilizes plant miRNAs via 3' terminal methylation. Most recently, we have focused on phased, secondary siRNAs, like tasiRNAs, and their diverse roles in plant gene and genome regulation. I will discuss our recent data and methods, and the insights that these provide into the rich populations of small RNAs generated from plant genomes.

SYM-07-02

CELL TYPE SPECIFIC DNA METHYLOMES OF THE ARABIDOPSIS ROOT

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Methylation of cytosines within the genome of many eukaryotic organisms is an essential process for development, transcriptional regulation, and genome defense. Within the Arabidopsis genome, DNA methylation plays a critical role in suppression of transposable elements, while its role in cell type specific transcriptional regulation remains poorly understood. To date, most comprehensive studies of this modification have analyzed heterogeneous populations of cells within a complex tissue, complicating the characterization of any cell-type specific DNA methylation patterns. Here we present whole-genome single-base resolution DNA methylomes, in addition to mRNA and small RNA transcriptomes, for several distinct cell types isolated from the Arabidopsis root tip. We identified numerous discrete loci within the genome that were differentially methylated between cell types, in addition to global differences in methylation in a subset of the cell types analyzed. Integration of the DNA methylome maps with transcriptome profiles further enables analysis of the potential role of differential DNA methylation on cell type specific patterns of gene expression. Overall, these genome scale maps provide new insights into the fine scale modulation of DNA methylation between distinct cell types within complex plant tissues.

SYM-07-03

PLANT VIRAL MICRORNA-LIKE SMALL RNA TARGETS HOST DEFENSE GENE THROUGH DCL1-DEPENDENT RNAI PATHWAY

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MicroRNAs encoded by several human, animal and insect viruses have been shown to suppress host gene expression and increase viral replication. Viral small interfering RNAs (vsiRNA), originating from plant RNA viruses as a result of viral RNA degradation, has been shown to interfere with host genes. Although, indirect effects have been confirmed for DICER-like-1 (DCL-1) in this pathway but no direct association of DCL-1 has been established, so far, with the viral siRNA. Here, we report that small RNA encoded by the positive-strand plant RNA Potyvirus, Turnip mosaic virus (TuMV) harnesses plant host DCL1-dependent RNAi pathways in a similar manner than microRNAs. We identified the localization of hairpin precursor for this small RNA in the nucleus, while the mature viral small RNA produced mainly by DCL2 and also by DCL4 accumulated in the cytoplasm. This small RNA targets anti-viral defense in Arabidopsis by specifically suppressing the expression of the abscisic acid-inducible gene HVA22d. HVA22d is known to be active during abiotic stress responses and our results show that it also has a significant role in viral defense in the plant host. These findings indicate that plant RNA viruses may encode and employ microRNA-like small RNAs in a manner distinct from plant microRNAs but exploiting the host RNAi machinery. This introduces a new complexity in the plant-virus arms race, which may enable us to develop new strategies for virus resistance.

SYM-07-04

NON-ADDITIVE GENE EXPRESSION AND EPIGENETIC INSTABILITY IN ARABIDOPSIS HYBRIDS

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We have employed transcriptome sequencing and ultra-high throughput SNP genotyping in Arabidopsis allopolyploids using next generation sequencing to determine the contribution of homeoallele bias to non-additive gene expression in Arabidopsis hybrids. High density coding region SNP and deep transcriptome coverage enabled us to discover that homologous genes with a biased transcript accumulation in favor of one homeoallele are no more likely to be non-additively expressed than those genes without such a bias. The source of this non-additive gene expression is still unclear, but likely stems from cis- and trans-regulatory divergence between the progenitor-derived chromosome sets. Although some parental bias responsible for non-additive gene expression is inherited in cis, we conclude that non-additive gene expression in Arabidopsis hybrids is largely a trans- effect consistent with the Bateson-Dobzhansky-Muller hypothesis of gene interaction in hybrids. In contrast, genomic DNA and the transcriptome and small RNA sequence analysis showed that differences between paternal and maternal small interfering RNA (siRNA) are responsible for epigenetic instability in transposon sequences. This parent-specific difference in small RNA leads to epigenetic reprogramming in hybrids and heritable transcriptional activity at sequences that are normally transcriptionally silent in the parents, which only subsides after several generations of inbreeding.

SYM-08-01

REGULATION OF PEROXISOMAL AND MITOCHONDRIAL DYNAMICS IN ARABIDOPSIS

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Plant peroxisomes and mitochondria are essential and highly dynamic organelles that act coordinately in a number of metabolic pathways crucial to energy production and metabolism. Research in our group focuses on elucidating the mechanisms that govern the dynamic behavior of these energy organelles and relating organelle dynamics to changes in plant physiology and development. Using a combination of molecular genetics, cell biology, biochemistry and proteomics, we have identified proteins involved in the multiplication of peroxisomes and mitochondria as well as proteins that regulate peroxisomal function through proteolysis. In Arabidopsis, the division of these two types of organelles is controlled by shared factors such as dynamin-related protein 3 (DRP3), its anchor FISSION1 (FIS1), and the plant-specific protein Peroxisome and Mitochondrial Division factor 1 (PMD1), which functions independently from DRP3 and FIS1. The function of some of these key mediators of mitochondrial and peroxisomal division is regulated post-translationally. For example, protein phosphorylation can inhibit the function of DRP3. Cardiolipin (CL), a phospholipid that is primarily, if not exclusively, localized to mitochondria, promotes mitochondrial division by stabilizing the higher order protein complex of DRP3. At the protein degradation side, our studies suggest that the Arabidopsis RING peroxins PEX2, PEX10, and PEX12 can function as E3 ligases and act together with the ubiquitin receptor protein DSK2 in the peroxisome membrane-associated protein degradation system.

SYM-08-02

CHLOROPLAST PROTEIN BIOGENESIS

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The evolution of chloroplasts from endosymbiotic cyanobacteria profoundly affected the biology of eukaryotic cells. In this process, two key events are thought to be essential: the transfer of genetic materials from the endosymbiont to the host nucleus, and the establishment of protein targeting mechanisms to the endosymbiont. Phylogenetic analysis of chloroplast constituents supports the endosymbiotic gene transfer. For the protein targeting mechanisms, AKR2A/B, two highly homologous ankyrin repeat domain (ARD)-containing proteins, serve as cytosolic factors in protein targeting to chloroplast outer membrane (COM). However, it is still mysterious how the protein targeting mechanisms were established during the organellogenesis. We have investigated how chloroplast proteins are specifically targeted to the chloroplasts. AKR2A evolved as a cytosolic targeting factor for COM proteins from the ARD of the host cell by a stepwise addition of the N-terminal domains, and two lipids monogalactosyldiacylglycerol (MGDG) and phosphatidylglycerol (PG) were selected from the endosymbiont as a chloroplast-localized AKR2A receptor for the protein targeting. Structural analysis, molecular modeling and mutational analysis of the ARD identified two adjacent sites for the simultaneous and synergistic binding of MGDG and PG. AKR2A mutants defective in MGDG and/or PG binding showed impaired chloroplast binding. Furthermore, Arabidopsis mgd1-3 and pgp1-1 mutants with lower levels of MGDG and PG in chloroplasts, respectively, had defects in AKR2A binding to chloroplasts and targeting of COM proteins. In addition, I will present evidence that AKR2A directly interacts with ribosome to capture its cargo during translation.

SYM-08-03

A MECHANISM FOR LOCALISED LIGNIN DEPOSITION IN THE ENDODERMIS

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The precise localisation of extracellular matrix and cell wall components is of critical importance for multicellular organisms. Lignin is a major cell wall modification that often forms intricate subcellular patterns that are central to cellular function. Yet, the mechanisms of lignin polymerisation and the subcellular precision of its formation remain enigmatic. Here we show that the Casparian strip, a lignin-based, paracellular diffusion barrier in plants, forms as a precise, median ring by the concerted action of a specific, localised NADPH oxidase, brought into proximity of localised peroxidases through the action of CASPARIAN STRIP DOMAIN PROTEINS (CASPs). Our findings in Arabidopsis provide a simple mechanistic model of how plant cells regulate lignin formation with subcellular precision. We speculate that scaffolding of NADPH oxidases to the downstream targets of the reactive oxygen species (ROS) they produce might be a widespread mechanism to ensure specificity and subcellular precision of ROS action within the extracellular matrix.

SYM-08-04

CALMODULIN-MEDIATED CALCIUM REGULATION IN PLANT ORGANELLES

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Calcium is an important second messenger and there is increasing evidence that plant organelles are integrated into the calcium signalling network of the cell. We have shown that calcium/calmodulin affects the transport of nuclear-encoded proteins into chloroplasts and plant mitochondria. Calcium regulation of protein import into chloroplasts is mediated by calmodulin-binding to Tic32, a component of the redox regulon of the import machinery, suggesting a cross-talk between different signaling pathways. Organellar calmodulin targets further include two AFG1-like AAA-proteins, one of which was suggested as a component of the mitochondrial stress response (van Aken et al. 2009). Our studies show that AFG1L1 and AFG1L2 have acquired a calmodulin-binding domain not present in AFG1-like proteins from yeast or humans. A significant number of potential calmodulin targets have been identified in different plant organelles, further corroborating a strong expansion of this regulatory network across the whole plant cell but also necessitating the presence of corresponding calmodulins in these organelles. The Arabidopsis genome indeed encodes over 50 calmodulins and calmodulin-like proteins, many of which contain potential targeting sequences. Our systematic approach to analyse their subcellular localization has already yielded calmodulin-like proteins targeted to mitochondria and peroxisomes and we recently identified two calmodulin-like proteins localized to the endomembrane system. We have furthermore generated a set of transgenic plant-lines targeting aequorin to the inside of several organelles as well as their cytosolic membrane surfaces. These plant lines are currently utilized to analyse organellar calcium changes in response to environmental stress and plant development.

SYMPOSIUM 9 – EMERGING TECHNOLOGIES AND SYSTEMS BIOLOGY

Sponsored by Annals of Botany

SYM-09-01

MAPPING SPATIOTEMPORAL GENE REGULATORY NETWORKS GUIDING ROOT VASCULAR DEVELOPMENT

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Arabidopsis root development provides a remarkably tractable system to delineate cell type-specific, developmental gene regulatory networks and to study their functionality in a complex multicellular model system over developmental time. We present gene regulatory networks guiding two aspects of vascular cell type development, specifically xylem cell specification and differentiation and vascular proliferation. Two components of transcriptional regulation are elucidated - transcription factor-mediated regulation and Polycomb Repressive Complex 2 (PRC2)-mediated regulation. Together, these networks identify novel regulators of vascular development and provide considerable insight into the combinatorial nature of root development at cell type and temporal stage-resolution.

SYM-09-02

CHROMATIN DYNAMICS AND CELL FATE SPECIFICATION IN ARABIDOPSIS

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How can a single genome be programmed to produce each of the cell types of a multicellular organism? This is a fundamental question in epigenetics and development, but one that has been difficult to address due to the technical challenges associated with epigenomic analysis of individual cell types in a developing organism. To make this problem more tractable, we developed a method called INTACT (isolation of nuclei tagged in specific cell types) that allows the examination of transcriptome profiles and chromatin features of specific cell types within a tissue. This approach was used to study the differentiation of the two epidermal cell types of the Arabidopsis root, hair cells and non-hair cells, which arise from a common progenitor stem cell. Our analysis revealed hundreds of genes that are specifically expressed in each cell type, and showed that cell type-specific gene expression is often associated with characteristic chromatin differences between cell types. Specifically, preferential expression of a gene in one cell type often correlates with major differences between the cell types in the trimethylation of histone H3 at lysines 4 and 27. This suggests that the balance between polycomb- and trithorax-group protein mediated histone methylation plays an important role in establishing cell type-specific transcriptional output during root epidermal cell fate specification. Current work is aimed at identifying a larger suite of chromatin-based gene regulatory mechanisms that underlie genome reprogramming during cell differentiation and organ formation. We are also expanding our transcriptome and epigenome analyses to other plants and cell types, as well as testing hypotheses generated from these data.

SYM-09-03

A HIGH-RESOLUTION GENE EXPRESSION MAP OF ARABIDOPSIS SHOOT APEX

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The shoot apical meristem (SAM) of land plants, harbors stem cells at their tip. Stem cell self-renewal and their differentiation in to distinct cell types take place within the SAM stem cell niche. Although a few key factors have been identified in this microenvironment, which are involved in stem cell maintenance and differentiation. However, the regulatory mechanisms underpinning transition of stem cells in to differentiated cell types are not elucidated. Our current understanding of gene expression patterns of differentiating stem cell daughters at single resolution is still lacking. We have identified and successfully developed fluorescent reporters for 10-distinct cell types of Arabidopsis SAM stem cell niche. We isolated cells from distinct cell layers (epidermal / L1 layer, sub-epidermal / L2 layer and L3 layer /corpus) as well as from differentiating stem cell progenitors from radial domain of SAM using fluorescent activated cell sorter and employed whole genome microarrays to record their gene expression at single cell type resolution. Microarray data analysis revealed several important gene regulatory networks related to plant development, stress physiology and metabolism. This data set will be quite useful in unraveling the mechanism of cell identity transition during stem cell differentiation in the SAM.

SYM-09-04

BORDER CONTROL - THE MEMBRANE-LINKED INTERACTOME OF ARABIDOPSIS

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Cellular membranes control solute transport and act as signaling platforms. Receptors, transporters and enzymes in lipid bilayers communicate with and are regulated by intracellular signaling networks via protein-protein interactions. To systematically identify interactions, we performed a binary interaction screen of Arabidopsis membrane and signaling proteins using the split ubiquitin system in yeast. We have identified a high confidence network containing over 10,000 interactions, covering a test space of ~6.4 million pairs. While many expected interactions were confirmed, e.g. between half-ABC transporter domains and between aquaporin dimers, the vast majority of interactions were novel, including an overrepresented set involving proteins of unknown function. Among potential regulatory associations in membrane protein trafficking, turnover, and phosphorylation were interactions between receptor-like kinases and trafficking proteins or small GTPases. Observed interactions also led us to identify the regulation of peptide mobilization transporters by abscisic acid receptor components. The interactions discovered represent a critical expansion of knowledge and serve as a resource for gene discovery and hypothesis generation.

SYMPOSIUM 10 – ENERGY BIOLOGY AND METABOLISM

Sponsored by the ARC Centre of Excellence in Plant Energy Biology

SYM-10-01

THE EVOLUTION OF SIGNALLING PROTEINS FROM ENZYMES

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Plant genomes frequently contain multiple genes coding for a particular enzyme. The duplicated genes may be differentially regulated or encode enzymes isoforms with different properties and/or sub-cellular locations. Alternatively, the duplicated genes may become specialized and adopt new, non-enzymatic functions. The nine-member β-amylase (BAM) gene family in Arabidopsis is a good example of this. β-Amylase is known as a key enzyme involved in starch degradation. In Arabidopsis, starch is accumulated during photosynthesis in chloroplasts, serving as a transitory store of carbohydrate for use during the night. β-Amylases liberate maltose from starch and mutations causing a deficiency in chloroplastic β-amylase block starch breakdown. Surprisingly, two Arabidopsis β-amylase-like proteins, BAM7 and BAM8, are nuclear-localized and share an amino-terminal DNA-binding domain with a family of plant-specific transcriptional regulators involved in brassinosteroid signalling. Our data show that BAM7 and BAM8 function as transcription factors, binding a specific cis-regulatory element. Their deregulation alters patterns of gene expression, plant growth and development, and brassinosteroid sensitivity, but does not affect starch metabolism. Homologous genes have been identified in other plants including gymnosperms, and angiosperms (both monocot and dicot species), implying that their functional specialization occurred early in higher plant evolution. We are exploring the functions of these proteins. We hypothesize that the duplication of β-amylase genes facilitated the evolution of metabolic sensors that provide a regulatory link between carbon availability and growth control.

SYM-10-02

XYLOSE METABOLISM IN ARABIDOPSIS

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Xylan is a major component of the plant cell wall and is mainly comprised of the monosaccharide xylose. The plant cell has a range of mechanisms to deal with remobilized free sugars as a result of processes such as cell wall restructuring. Most free sugars within the plant cell are converted into their corresponding nucleotide sugars. The fate of xylose is less clear, although there is good evidence to suggest a salvage pathway through the pentose phosphate pathway via xylose isomerase. Recently we identified the only annotated Arabidopsis xylose isomerase enzyme (AT5G57655) in Golgi purified fractions. Xylose isomerase catalyses the isomerisation of xylose to xylulose and has been functionally characterized in bacteria, fungi and indirectly in plants nearly two decades ago. Given the enzymes association with Golgi membranes, we sought to re-examine the function and role of this enzyme in plants. We have used yeast complementation, promoter-GUS and transient localization analyses to confirm the enzymes function, expression patters and subcellular localization. We have also sought to confirm the recycling pathways in Arabidopsis using stable istopes, Arabidopsis mutants and metabolite analysis by LC-MS/MS. Finally virus induced gene silencing in tobacco is being used to determine the effect of gene silencing on this pathway.

SYM-10-03

REPRESSION OF FOLYPOLYGLUTAMATE SYNTHETASE ALTERS LIGNIN COMPOSITION AND CELL WALL DIGESTIBILITY IN ARABIDOPSIS

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Tetrahydrofolate and its derivatives, collectively referred to as folates are essential for several metabolic pathways including the phenylpropanoind pathway by facilitating the addition and removal of one-carbon (C1) units. Previously, we have shown that a mutation in the plastidial isoform of folylpolyglutamate synthetase, fpgs1 can be detrimental for postembryonic root development. Now, we have critically analyzed fpgs1 mutants in the aerial tissues. FPGS1 is strongly expressed in the vascular tissues, especially in the xylem parenchyma and tracheary elements. GeneChip data and real-time PCR analysis revealed that transcripts of several s-adenosyl methionine dependent methyl transferases, o-methyltransferases, and other genes involved in supplying methyl units to the phenylpropanoid pathway were also altered in the fpgs1 mutant. Furthermore, significant reduction in guaiacyl (G) lignin monomers were detected, while syringyl (S) lignin or p-hydroxyphenyl (H) lignin were not changed in fpgs1 compared to wild-type plants. Consequently, an increased digestibility and higher saccharification efficiency of the cell wall in the fpgs1 mutant was observed. The observed alterations in the cell wall chemistry in fpgs1 mutants underscore the importance of folylpolyglutamates in the highly compartmentalized C1 transfer reactions and its association with lignin biosynthesis. Our results provide genetic evidence that FPGS1 can be considered as a new target for cell wall improvement in biofuel production.

SYM-10-04

REQUIREMENT FOR THE PLASTIDIAL OXIDATIVE PENTOSE PHOSPHATE PATHWAY FOR NITRATE ASSIMILATION IN *ARABIDOPSIS*

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Sugar metabolism and the oxidative pentose phosphate pathway (OPPP) are strongly implicated in N assimilation although the relationship between them and the roles of the plastidial and cytosolic OPPP have not been established genetically. We isolated a mutant of the plastid-localized OPPP enzyme 6-phosphogluconolactonase 3 (6PGL3). 6pgl3-1 plants exhibited relatively greater resource allocation to roots but were smaller than wild type. They had lower content of amino acids and free NO3- in leaves than the wild type, despite exhibiting comparable photosynthetic rates and efficiency, and normal levels of many other primary metabolites. When N-deprived plants were fed via the roots with $^{15}NO_3^-$, 6pgl3-1 exhibited normal induction of OPPP and nitrate assimilation genes in roots, and amino acids in roots and shoots were labeled with ^{15}N at least as rapidly as in the wild type. However when N-replete plants were fed via the roots with sucrose, expression of specific OPPP and N assimilation genes in roots increased in the wild type but not in 6pgl3-1. Thus, sugar-dependent expression of N assimilation genes requires OPPP activity and the specificity of the effect of the 6pgl3-1 mutation on N assimilation genes establishes that it is not the result of general energy deficiency or due to accumulation of toxic intermediates. We conclude that expression of specific nitrate assimilation genes in the nucleus of root cells is positively regulated by a signal emanating from OPPP activity in the plastid.

SYM-11-01

CENTROMERES AND PARENTAL GENOME CONFLICT

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Centromeres are chromosomal landmarks that mediate accurate segregation of chromosomes during cell division. Centromeres are epigenetically determined by the unique deposition of a centromere specific histone variant(CENH3), which replaces canonical histone H3 in a subset of nucleosomes of centromeric chromatin. Using the plant model Arabidopsis thaliana, we have shown that in zygotic mitosis, centromere differences between a parent expressing hypomorphic CENH3 variants can induce genetic conflict with other parental genome that expresses wild-type(WT) CENH3. As a consequence, a massive genome catastrophic event is triggered during embryogenesis leading to an aberrant cell division process resulting in either partial or total elimination of the parental genome expressing hypomorphic CENH3, producing viable aneuploid and uniparental WT haploid embryo respectively. Characterization of the aneuploids revealed massive structural chromosomal aberrations such as deletion, duplication and translocation events suggestive of a complex chromosomal rearrangement(CCR) process. Preliminary evidence suggests that this rearrangement might be due to chromosome fragmentation event initiated during the genome elimination process, followed by an aberrant DNA repair event mostly involving non-homologous end joining(NHEJ) of the broken DNA fragments. This phenomena resembles a recently discovered chromosomal rearrangement mechanism observed in cancer cells called chromothripsis. Since most aneuploids harboring such complex chromosomal rearrangements are viable and produce progeny, this makes Arabidopsis an ideal model to understand the molecular events that initiate chromothripsis, a poorly understood process in cancer cells.

SYM-11-02

SIMON CHAN'S THREE QUESTIONS

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The faithful inheritance of eukaryotic chromosomes is dependent on centromere's ability to nucleate the formation of the kinetochore complex, which in turn mediates dynamic interaction with the spindle apparatus. Centromeric function is an epigenetic property whose mechanism of determination is unclear, but requires the presence of a histone variant, CENH3. Working with Arabidopsis thaliana, Simon Chan and coworkers have discovered that centromeres organized by a modified CENH3 are stable in crosses between similarly modified plants. However, when confronted in the zygote with centromeres determined by wild-type CENH3, the centromeres with the altered CENH3 cause failure of maintenance of the connected chromosomes, genome elimination and formation of uniparental haploids. Three questions were prominent in Simon's mind: 1. What are the consequences of CENH3 rapid evolution and can these mediate genome elimination in interspecific crosses? 2. What molecular and epigenetic mechanisms results in the elimination of the genome marked by the altered CENH3? 3. What are the genomic consequences of elimination? Aneuploids are formed in addition to haploids. Could these be the remnants of partially failed rescue? Three postdoctoral researchers joined Simon to address these questions: Shamoni Maheshwari, Mohan Marimuthu and Han Tan. Less than a year after Simon's death, their effort has produced substantial insight in the process. Their progress will be presented.

SYM-11-03

EXPLOITING CRYPTIC GENETIC VARIATION IN ARABIDOPSIS

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Wild strains of Arabidopsis thaliana display extensive variation in a variety of phenotypes and thus provide an excellent genetic resource to study the molecular basis of phenotypic variation. We have been exploiting natural variation in thermal responses of Arabidopsis thaliana at phenotypic and molecular level. Higher temperatures often reveal cryptic phenotypes and characterizing these unusual phenotypes results in identification of novel genes and pathways. One such cryptic phenotype resulted in the identification of a triplet repeat expansion, which provides an excellent model to study fundamental mechanisms associated with genetic diseases caused by such expansions in humans. I will discuss our recent progress on the associated genes and mechanisms.

SYM-11-04

SPATIAL AND TEMPORAL DYNAMICS OF DNA METHYLATION AND ITS EFFECTORS DURING SEXUAL REPRODUCTION IN *ARABIDOPSIS THALIANA*

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Sexual reproduction is a complex event involving the development of highly specialized cells, the gametes, which, upon fusion, produce a totipotent zygote. During this process, genome integrity and gene expression need to be properly regulated to ensure correct development of the next generation plant. In Arabidopsis, DNA methylation is believed to be stably maintained from one generation to the next but its regulation throughout plant development is largely unknown. We found that female gametogenesis is associated with a strong reduction of maintenance DNA methyltransferase expression and the preferential expression of de novo methyltransferases in the embryonic lineage. After fertilisation we observed a strong asymmetry between the two-fertilisation lineages i.e. the endosperm and the embryo. In particular, strong expression of de novo DNA methyltransferases leads to increased de novo methylation in the embryo, which is further maintained in the adult plant. de novo DNA methylation is directed by small RNAs via a process known as RNA-directed DNA methylation (RdDM). We have surveyed the expression of RdDM Argonaute (AGO) effector proteins during the reproductive process and describe here their specific enrichment and subcellular localizations in gametes. The patterns uncovered suggest that dynamic DNA methylation and RdDM allow the correct transmission of epigenetic information and simultaneously provides a window for adaptation.

SYM-12-01

BIOSENSORS FOR RECORDING TRANSPORTER AND ENZYME ACTIVITIES IN PLANTS

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We can measure ion and metabolite composition using mass spectrometry, we can determine regulation of transcription at the cellular level, we can localize proteins using fluorescent protein fusions and, with the advent of genetically encoded fluorescent biosensors (i.e. FRET sensors), also study the dynamics of ions and metabolites with cellular and subcellular resolution. The FRET sensors can be used to map analyte levels and dynamics in live plants, e.g. with the help of the RootChip, a microfluidic platform that enables multiparallel analysis of FRET changes in roots over days. The FRET sensors have successfully be used to identify missing functions and genes, e.g. the SWEET sugar transporters. The FRET sensors provide a unique opportunity to visualize and quantify the changes in sugar distribution in mutants as well as effects that are a consequence of pathogen infection. However, one key tool has still been missing, namely the ability to monitor the activity of proteins in real time in live tissues. Such a technology would allow us to monitor processes such as transport in individual cells, or observe the effect of regulation in real time, and to measure processes that are otherwise inaccessible, e.g. subcellular transport. We have exploited environmentally sensitive fluorophores to develop prototype activity sensors for important plant transporters. The development and utility of multiple such sensors will be presented.

SYM-12-02

LIVE-CELL STUDY OF CELL-TO-CELL COMMUNICATION IN POLLEN TUBE GUIDANCE

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Pollen tubes guidance is a good model to study cell-to-cell communication. Many male and female extracellular signaling molecules are involved in precise control of growth direction of the pollen tube, and real-time analysis is possible to examine the response of the pollen tube to extracellular stimuli. We have been using two model plant species, Torenia fournieri and Arabidopsis thaliana. Defensing-like peptide LUREs are pollen tube attractants of these species working in a short distance (a few hundred micrometers), which are secreted by two synergid cells on the side of the egg cell (Higashiyama et al., 2001, Science; Okuda et al., 2009, Nature; Takeuchi and Higashiyama, 2012, PLoS Biol.). To understand the molecular mechanism of pollen tube guidance, we have been taking two approaches of live-cell study (for review, Kurihara et al., 2013, Cell Growth Differ.). The first approach is to use precisely defined in vitro system, including development of various microfluidics devices by our engineering team (e.g., Horade et al., 2012, Proc. MicroTAS). Recent in vitro studies lead to discovery of novel intercellular signaling molecules involved in competency control of pollen tubes and long-distance attraction (a few millimeters). The second approach is based on in vivo imaging. We have shown that pollen tube guidance is intimately related with double fertilization (Hamamura et al., 2011, Curr. Biol.; Kasahara et al., 2012, Curr. Biol.; Maruyama et al., in revision). In this symposium, I will show our recent results of identification of novel signaling molecules and in vivo analysis of blocking of supernumerary pollen tubes.

SYM-12-03

THE IDA PEPTIDE REGULATES FLORAL ABSCISSION AND TRIGGERS AN OXIDATIVE BURST BY DIRECT BINDING TO THE HSL2 RECEPTOR

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Small peptides play an important role in plant growth and development. INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) encodes a putative peptide ligand necessary for the regulation of floral organ abscission. IDA is dependent on the receptor-like kinases (RLK) HAESA (HAE) or HAESA-LIKE 2 (HSL2) to exert its function (Stenvik et al. 2008, Cho et al.2008). Genetic interaction studies indicate that IDA signaling activates a MITOGEN-ACTIVATED PROTEIN KINASE cascade to induce separation between abscission zone (AZ) cells (Cho et al.2008). By performing a suppressor screen on ida mutant seeds the homeobox transcription factors, KNOTTED-LIKE FROM ARABIDOPSIS THALIANA1 (KNAT1), KNAT2 and KNAT6 were identified as downstream components of the IDA pathway (Shi et al. 2011). Here additional candidates from the suppressor screen identified by ullimina sequencing will be presented. IDA belongs to a family of IDA-LIKE (IDL) proteins that exhibit sequence diversity with the exception of a conserved 20 amino acid (aa) C-terminal domain that contains the functional part. Here we show that production of reactive oxygen species (ROS) is involved in floral abscission in Arabidopsis and that a dodeca IDA peptide with a hydroxyproline modification induces a rapid release of ROS in an oxidative burst assay upon binding and activation of HSL2. These results, showing direct biochemical interaction between IDA and HSL2, substantiate that these proteins form a peptide-receptor system regulating cell separation in the floral organ abscission zone.

SYM-12-04

CELL-TO-CELL SIGNALING MEDIATED BY MOBILE TRANSCRIPTION FACTORS IN THE ROOT VASCULAR TISSUE PATTERNING

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Cell type patterning and specification in multicellular organs requires extensive communication between cells. In the Arabidopsis root, vascular tissues, composed of xylem, phloem and procambium, are organized in a bisymmetric manner. Such organization is established very early in the root apical meristem. We previously showed that SHORTROOT transcription factor, generated from the subset of vascular cells, regulates the patterning of xylem vessels by moving into the endodermis. SHORTROOT does this by turning on the expression of mobile microRNA 165/6, which subsequently sets up the distribution of target HD-ZIP III transcription factors, dosage-dependent regulators of xylem vessels. Here, we present novel cases of cell-cell signaling mediated by mobile transcription factors in the root vascular patterning. In the case of xylem development, we found that the movement of AHL4, an AT-hook family transcription factor, from the procambium to the xylem precursor cells is critical for the establishment of xylem boundary. Without this movement, xylem domain expands into the procambium. During phloem development, we discovered that the SHORTROOT moving from the procambium into the phloem initial regulates the sieve cell formation. SHORTROOT in the phloem initial drives the asymmetric cell division for the formation of proto- and metaphloem sieve cells. We further used a combinatorial genomics approach to find potential downstream regulators of SHORTROOT in this process. Among several candidates, we identified that a NAC domain transcription factor, activated by SHORTROOT in the phloem, promotes the asymmetric cell division for the phloem sieve cells. Taken together, these findings further demonstrate that intercellular movement of transcription factors provides essential signals for the tissue patterning in plants.

SYMPOSIUM 13 – TRANSGENERATIONAL INHERITANCE

Sponsored by Monsanto Company

SYM-13-01

TRANSGENERATIONAL EPIGENETIC INHERITANCE IN *ARABIDOPSIS*

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Until recently, a key assumption in biology has been that mutations in the DNA sequence are the only source of heritable phenotypic diversification and therefore that adaptation is impossible in the absence of DNA sequence variants. However, this view is being increasingly challenged by the observation that changes in chromatin states, which are pivotal for the control of genome activity in eukaryotes, can occasionally become locked in and inherited across multiple sexual generations, independently of any change in the genome sequence. This system of inheritance, called epigenetic, is best documented in plants and often involves differential DNA methylation of repeat sequences, notably transposable elements. However, there is still a lack of systematic studies that experimentally examine the stability of epigenetic changes and test their consequences on genome function as well as their ecological or evolutionary relevance, which could be different from that of DNA sequence variants, notably by providing a more rapid and reversible route to adaptation. I will present our efforts at answering some of these questions using a population of near-isogenic, epigenetic Recombinant Inbred Lines (epiRILs) in Arabidospsis.

SYM-13-02

EPIGENETICS IN HYBRIDS

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Epigenetic systems play a critical role in development through regulating a number of biological processes including silencing of transposable elements, regulating gene expression and controlling genomic imprinting. An important developmental phenomenon is the increased growth frequently obtained in F1 offspring (heterosis). Although the frequency of heterosis is correlated with genetic diversity of the parents, significant heterotic events can still occur if the parents are genetically very similar. In such cases, the required allelic diversity between parents may be epi-allelic. To test this we examined two major epigenetic components, DNA methylation and siRNA profiles, of intraspecific hybrids between Arabidopsis accessions C24 and Landsberg erecta. Despite being highly genetically similar, C24 and Ler show substantial epi-allelic variability across the genome. These parental epigenetic profiles change substantially when combined into the same nucleus, with the F1 hybrids showing decreasesin 24nt siRNAs at loci where parental expression levels of siRNAs are markedly different. This correlates with alterations to the hybrid's methylome at loci where the parental methylation levels are different. Two main processes, Trans-Chromosomal Methylation (TCM) and Trans-Chromosomal deMethylation (TCdM), were identified as causing the majority of changes to methylation in the hybrids. These two processes involve one parental allele inducing a change in the methylation status of the corresponding parental allele. As siRNA direct site-specific methylation, can act in trans and are frequently associated with TCM and TCdM events, it is likely siRNA are a key signalling molecule driving TCM and TCdM events. The altered epigenetic state of the hybrids can lead to changes in gene expression and therefore we believe that epigenetic diversity provides allelic transcriptional variability contributing to the frequency and magnitude of heterosis obtained in F1 hybrids.

SYM-13-03

RECONSTRUCTING *DE NOVO* SILENCING OF AN ACTIVE PLANT RETROTRANSPOSON: DYNAMICS, MECHANISMS, BIOLOGICAL IMPLICATIONS

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Retrotransposons are molecular parasites structurally and functionally similar to retroviruses that comprise a major portion of plant genomes, contributing to their size and organization during evolution via successive proliferation/deletion 'bursts'. Transposons are mostly maintained in a quiescent state through transcriptional gene silencing (TGS), which may be temporarily reversed, leading to bursts of retrotransposition. However, how invasive TEs are detected and silenced de novo remains largely unknown. By releasing TGS from the endogenous LTR-retrotransposon Évadé (EVD) in Arabidopsis, we have deciphered the timing, spatial distribution and mechanisms underpinning the proliferation and eventual silencing of EVD. Once transcriptionally active, EVD displays a very defined expression pattern that contributes to progressive increase in copy number and expression levels through generations and is subjected to the same small interfering RNA (siRNA) pathway that targets viruses for post-transcriptional gene silencing (PTGS). Both developmental and molecular features of EVD biology, including its remarkable ability to evade host antiviral RNA-interference, ultimately lead to its silencing over multiple generations. This is achieved at a threshold of □40 copies/genome, at which a switch from PTGS to TGS leads to the production of abundant 24-nt siRNAs from the retrotransposon promoter, directing the DNA methylation machinery to silence all of EVD new copies at the transcriptional level. The underlying processes are accompanied by widespread diversification of the Arabidopsis genome and de-novo epiallelism creating an extensive reservoir of selectable and potentially adaptive traits.

SYM-13-04

EPIGENETIC TRANSGENERATIONAL RESPONSE TO ABIOTIC STRESS: A PREDICTIVE ADAPTIVE RESPONSE PRIMING STOMATA

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Transgenerational inheritance of abiotic stress-induced epigenetic modifications in plants has potential adaptive significance. When we examined inheritance of low Relative Humidity (RH)-induced DNA methylation for loci in the stomatal developmental pathway in Arabidopsis we found that parental low RH-induced DNA methylation and stomatal phenotype were heritable, but this was reversed in the progeny under repeated treatment. Our findings suggested a predictive adaptive response for stomatal development, where progeny stomatal frequency is set to match the parental growing environment in anticipation that this same environment will be encountered in the next generation. When an unpredicted environment is encountered (reduced or increased RH), methylation patterns and expression at the SPCH locus are similar and stomatal index is reduced, and vice versa when the environment fits the prediction. Both parents and their offspring are pre-conditioned by low RH to resist more severe water stress (periodic drought) and this priming is associated with the maintenance of methylation. The effect of matching or mismatching with the predicted environment transgenerationally outweighs the effect of present stress on methylation during growth and may help to explain the flexibility of transgenerational epigenetic inheritance.

SYM-14-01

A MEMBRANE BOUND NAC TRANSCRIPTION FACTOR IS A REGULATOR OF MITOCHONDRIAL RETROGRADE REGULATION OF THE OXIDATIVE STRESS RESPONSE IN ARABIDOPSIS

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Upon disturbance of their function by stress, mitochondria can signal to the nucleus to steer the expression of responsive genes. This mitochondria-to-nucleus communication is often referred to as mitochondrial retrograde regulation (MRR). Although reactive oxygen species and calcium are likely candidates for MRR, the protein signaling components remain largely unknown. Through meta-analysis of transcriptome data, we detected a set of genes that are common and robust targets of MRR and used them as a bait to identify transcriptional regulators of MRR. In the upstream regions of these mitochondrial dysfunction regulon (MDR) genes, a cis-regulatory element, the mitochondrial dysfunction motif (MDM) was found that is necessary and sufficient for gene expression under various mitochondrial perturbation conditions. Yeast one-hybrid analysis and electrophoretic mobility shift assays revealed that transmembrane domain containing NAC transcription factors bound to the MDM cis-regulatory element. We demonstrate that MRR-induced expression of the MDR genes is mediated by a direct interaction of these NAC transcription factors with the MDM cis-regulatory element and triggers an increased oxidative stress tolerance.

SYM-14-02

SIGNALING THROUGH GABA-GATED ANION CHANNELS IS EVOLUTIONARILY CONSERVED BETWEEN ANIMALS AND PLANTS, AND HAS A KEY ROLE IN STRESS SIGNALING IN PLANTS

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It is well documented that mammalian gamma-aminobutyric acid (GABA)-gated ion channels are essential for proper nerve function. GABA also rapidly accumulates in plant tissues in response to various stresses, and has been shown to regulate plant cell growth. Until now it was thought that GABA could act in this way solely through the regulation of metabolic pathways as no molecular evidence had been found to the contrary. However, our data suggests that GABA can be a signal in plants as it is in animals, through its action as an ion channel ligand. We have identified a GABA-binding site within plant Aluminium-activated Malate Transporter (ALMT) proteins and demonstrate that anion flux through ALMT proteins can be regulated by GABA with an affinity in the low micromolar range. Site directed mutagenesis of this region alleviates GABA block but does not alter other channel properties. We show (see POS-TUE-243) that GABA regulation of wheat ALMT1 has a role in aluminum and alkaline pH tolerance. The ALMT family in Arabidopsis contains 14 members, with most of these genes having an unknown function. This talk will explore the role of GABA-gated ALMT proteins in both wheat and Arabidopsis, and will reveal how signaling mediated through GABA-gating anion channels is likely to have broad implications for both basic plant physiology and stress tolerance.

SYM-14-03

NIA1NIA2 MUTATION REGULATES ION HOMEOSTASIS AND NITRIC OXIDE-MEDIATED CONTROL OF GUARD CELL ION CHANNELS IN ARABIDOPSIS

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The water-stress hormone abscisic acid induces stomatal closure through an elevation of downstream signalling molecules such as hydrogen peroxide and nitric oxide (NO) along with subsequent changes in ion channels responsible for the loss of K^+ and anion. However, the effect of NO on the characteristics of guard cell ion transport is not demonstrated directly in any NO non-producing Arabidopsis mutants. Electrophysiology, whole-plant physiology, and quantitative RT-PCR were employed to dissect the interaction of nitrogen and potassium nutrition in regulating stomatal opening, CO_2 assimilation, K^+ uptake, K^+ channel activity, and KAT1 expression in Arabidopsis nitrate reductase mutant nia1nia2. We found that nia1nia2 mutation affects K^+ homeostasis through a general action on nitrogen assimilation in Arabidopsis. Also, ABA inhibited inward K^+ current ($I_{K,in}$) and ABA enhanced slow anion current (I_{anion}) in the guard cells of wildtype Col-0 were not observed in nia1nia2 mutant. In addition, guard cells of nia1nia2 plants only showed normal $I_{K,in}$ and I_{anion} response to ABA with exogenously supplied NO. Simulations with the OnGuard model suggested that intracellular Ca^{2+} release could be considered a possible mediator of the observed effects of reduced K^+ content and suppressed stomatal responsiveness, on the untested assumption that NO_3^- moves through the same pathways as Cl^-. In summary, our results confirm the role for NO as one of the critical signalling components in ABA-induced stomatal closure and reveal subtle and complicated interactions between potassium and nitrogen nutrition in Arabidopsis nia1nia2 mutant.

SYM-14-04

MUTUALLY EXCLUSIVE ALTERATIONS IN SECONDARY METABOLISM ARE CRITICAL FOR THE UPTAKE OF INSOLUBLE IRON COMPOUNDS BY ARABIDOPSIS AND *MEDICAGO TRUNCATULA*

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The generally low bioavailability of iron (Fe) in aerobic soil systems forced plants to evolve sophisticated genetic strategies to improve the acquisition of Fe from sparingly soluble and immobile Fe pools. To distinguish between conserved and species-dependent components of such strategies, we analyzed Fe deficiency-induced changes in the transcriptome of two model species, Arabidopsis thaliana and Medicago truncatula. Transcriptional profiling by RNA-sequencing revealed a massive up-regulation of genes coding for enzymes involved in riboflavin biosynthesis in Medicago and phenylpropanoid synthesis in Arabidopsis upon Fe deficiency. Coexpression and promoter analysis revealed that the synthesis of riboflavins and phenylpropanoids is tightly linked to and putatively co-regulated with other genes encoding proteins involved in Fe uptake. We further provide evidence that the production and secretion of phenolic compounds is critical for the uptake of Fe sources with low bioavailability, but dispensable under conditions where Fe is readily available. In Arabidopsis, homozygous mutations in the Fe(II)- and 2-oxoglutarate-dependent dioxygenase family gene F6'H1 and defects in the expression of PLEIOTROPIC DRUG RESISTANCE 9, encoding a putative efflux transporter for products from the phenylpropanoid pathway, compromised the uptake of a Fe source of low Fe bioavailability. It is concluded that that production and secretion of compounds that facilitate the uptake of Fe is an understudied but essential component of the reduction-based Fe acquisition strategy, which is likely to contribute substantially to the efficiency of Fe uptake in natural conditions.

SYMPOSIUM 15 – NATURAL VARIATION, EVOLUTION AND PHENOMICS II

Sponsored by the Australian Plant Phenomics Facility

SYM-15-01

PHENOTYPING TECHNOLOGIES FOR QUANTITATIVE ANALYSES OF ARABIDOPSIS SHOOT AND ROOT SYSTEMS

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The ability to quantify structural and functional plant traits at an accelerated pace is crucial to enable genome-phenome association, unravel genotypes x environment effects, and ultimately select plants with increased resource use efficiency (light, water, nutrients). The development and application in the lab and in the field of non- or minimally-invasive phenotyping methods provide new research opportunities to design assays and screening experimentation focused on leaf and root traits. In this presentation we will highlight the progress in the application of different imaging modes, from 2D color imaging to multi-spectral reflectance analyses and 3D reconstruction. Based on knowledge generated at the Juelich Plant Phenotyping Center, these methods will be discussed according to the measurements principles, theoretical and practical limitations, and robustness for implementation of systematic phenotyping in Arabidopsis and other model species. For each of the most important methodologies, for example color- and thermal- imaging and photosystem II fluorescence dynamics, we will present studies in Arabidopsis. These will include shoot and root phenotyping of key genetic resources, such as the 1001 Genome Arabidopsis sequencing project. In this context we have completed a first analysis of variability in growth and root and shoot architecture of 80 genotypes grown under non-limiting conditions and at low temperature. We conclude that non-invasive imaging methods coupled with automated hardware and software solutions will allow in the medium term more powerful association studies of both macroscopic and molecular phenotypes with underlying alleles and polymorphisms. At the same time, we caution that meta-analyses and comparison of large data sets across labs requires stricter criteria to characterize and report upon environmental and experimental meta-data.

SYM-15-02

PHENOTYPING PHOTOSYNTHESIS, BIOMASS AND GROWTH IN HIGH THROUGHPUT WITH MODEL PLANTS

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Transgenic and insertional mutants in Arabidopsis have been extensively used to investigate mechanisms controlling photosynthetic flux in higher plants. Mutants in electron transport (Reiske iron sulphur protein; Ferredoxin-NADP-oxidoreductase, Ferredoxin-thioredoxin oxidoreductase, PsbO) are now available either as gene suppression or insertional mutants and known to result in reductions in photosynthetic flux, the degree of which is dependent on growth conditions, in particular irradiance and the degree of reduction in the target protein. This paper describes the photosynthetic and growth dynamics of these mutants measured with image based plant Phenomics platforms at the High Resolution Plant Phenomics Centre which use a combination of colour, thermal, hyperspectral and pulse modulated chlorophyll fluorescence imaging. The aim of this experiment is threefold. First, the experiments determine the most effective protocols for detecting mutants in electron transport using pulse modulated chlorophyll fluorescence imaging and spectral reflectance; second, they explore the relationship between electron transport / photophosphorylation capacity and growth over the entire lifecycle of the plant and third, these mutants provide information on the relationship between stomatal behavior and photosynthetic electron transport given that the level of these proteins will most likely be affected in both mesophyll and guard cell chloroplasts.

SYM-15-03

GENETIC ARCHITECTURE OF DROUGHT TOLERANCE IN *A.THALIANA*

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Plants must constantly adjust to a plethora of biotic and abiotic stresses that markedly influence growth and development. Among abiotic stresses, drought in particular has a major influence on plant performance and agricultural productivity. Response to drought stress is complex and involves many genes with pleiotropic effects. Many components underlying this regulatory network have already been identified using mutants or transgenic plants. However, it is far from obvious whether these genes play a role under natural conditions. Genome-wide association studies (GWAS) are nowadays a standard approach to identify causal relationships between genotypes and phenotypes. The performance of 120 different Swedish A.thaliana accessions was analysed under control and stress conditions. Both macroscopic (i.e. growth rate) and molecular (RNA sequencing) phenotypes were collected. The data were analysed using not only standard single-trait GWAS, but additionally explicit GWA models of gene-by-environment interactions and models that incorporate RNA expression data. These analyses recovered previously known candidates, as well as novel association that give insight into the architecture of this adaptive trait. Interestingly most molecular differences observed are found rather in proteins linked to secondary metabolism than in key signalling components.

SYM-15-04

GENETIC BASIS OF GROWING SEASON ADAPTATION IN ARABIDOPSIS: PHENOMICS IN CLIMATE CHAMBERS

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Genetic variation is filtered by the regional and local environment through selection on adaptive traits. The genetic basis of growth and flower time has been dissected in a global sample across multiple simulated locations and spring and fall plantings. Loci were identified that sense changing local climates, control yield and are shaped by selection. New studies are recording gene expression, photosynthesis, and photomorphogenesis under multispectral LED lights simulating fluctuating sun and canopy shade, with diurnal and seasonal temperature and moisture control. This fine resolution developmental profiling is being coupled with photo thermal models to integrate phenotype, genotype, and environment.

SYM-16-01

MECHANISMS BY WHICH THE PAIRED IMMUNE RECEPTORS, RPS4 AND RRS1, FUNCTION IN *ARABIDOPSIS THALIANA*

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Successful pathogens secrete effector proteins to suppress immunity and cause disease in host plants. The effector-triggered immunity (ETI) is conferred by intracellular disease resistance (R) proteins. The R proteins often carry the conserved nucleotide-binding (NB) and leucine rich repeat (LRR) domains with variable N-terminal domains, coiled coil (CC) or toll interleukin 1 receptor (TIR). Understanding how R proteins are activated and initiate immune responses is one of the major questions in plant biology. Two Arabidopsis TIR-NB-LRR class R proteins, RPS4 (resistance to Pseudomonas syringae 4) and RRS1 (resistance to Ralstonia solanacearum 1), are required for recognition of AvrRps4 and PopP2 that are secreted by the bacterial pathogens Pseudomonas syringae and Ralstonia solanacearum, respectively. The molecular mechanisms by which RPS4 and RRS1 i) recognize two sequence-unrelated effectors and ii) activate transcription of defence genes are poorly understood. Interestingly, RRS1 carries WRKY DNA-binding domain (DBD) suggesting its role in transcriptional regulation of defence genes. The sensitive to low humidity (slh) 1 mutant carries single amino acid insertion at WRKY DBD of RRS1 that results in reduced DNA-binding property and causes lethal phenotype. The slh1 lethal phenotype is suppressed in high temperature or humidity growth condition. We set out an experiment to investigate how the paired R proteins, RPS4 and RRS1, function by screening 500,000 EMS-mutagenised slh1 population (M2) for the reduced lethal phenotype at low temperature. As a result, we isolated 103 sushi (suppressor of SLH1 immunity) mutants with varying degree of phenotypes. The detailed genetic and biochemical analysis of some sushi mutants that provide the mechanistic insights of dual R protein function and immune signalling in Arabidopsis will be presented.

SYM-16-02

ANALYSING PLANT DEFENCE RESPONSES IN ARABIDOPSIS WITH A FOCUS ON THE FUNGAL PATHOGEN, *RHIZOCTONIA SOLANI*

Singh K.B.[1, 2], Foley R.C.[1], Hane J.[1], Thatcher L.F.[1], Gleason C.[1] and Anderson J.P.[1, 2]
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Plant pathogens cause major losses to crops throughout the world. We have been using forward genetic screens, with stress-responsive promoters linked to the luciferase reporter gene in Arabidopsis, to gain insight into plant defence/stress gene expression. This approach has identified novel Arabidopsis mutants with altered responses to pathogens and pests. In a related approach we have set up Arabidopsis pathosystems to study various isolates of the economically important and broad host range, necrotrophic fungal pathogen, Rhizoctonia solani. Progress from using a combination of genetic and genomic approaches on both the plant and fungal side of the interaction will be presented, including the potential role of oxidative stress in resistance to R. solani AG8 in Arabidopsis.

SYM-16-03

CHEMICAL GENETIC ANALYSIS OF MAMP-TRIGGERED CALCIUM SIGNATURES

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In plants, transient changes of cytosolic calcium occur upon the perception of microbe-associated molecular patterns (MAMP). This MAMP-specific calcium signature is an essential early part of MAMP-triggered immunity (MTI). Using chemical genetics, we aim at elucidating the role of molecular components involved in the generation of calcium signatures and their involvement in MTI. I identified several protein kinase inhibitors (PKI), especially tyrosine kinase inhibitors, as potential tools for identification and characterization of such components. Receptor tyrosine kinase activity, although common and well studied in vertebrates, is a recent discovery in plants. Additionally, two of the identified PKI are mycotoxins, pointing towards a distinct physiological function of these compounds, possibly affecting plant microbe interaction. To further analyze the bioactivity of these compounds and identify their respective targets and mode of action, I am following two strategies. First of all, I have tested structural analogues of the candidates to establish structure-activity-relationships. This allows target identification via biochemical affinity labeling. Secondly, I am trying to pinpoint the protein targets of the bioactive compounds by using a collection of known calcium signaling mutants and site-directed mutations of known signaling components, transiently expressing the Ca2+-sensitive reporter in protoplasts. In conclusion, using both biochemical as well as genetic approaches, I am trying to identify novel molecular mechanisms and components constituting the calcium signaling apparatus. In particular, I have generated new tools for studying post-translational modification of kinases and its impact on calcium channel activation. Furthermore, these tools will aid in investigating the significance of the calcium signature during MTI and its impact on downstream signaling events.

SYM-16-04

MULTIPLE RESISTANCE PATHWAYS ELICITED BY TMV IN *N* GENE TOBACCO

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Hypersensitive resistance to tobacco mosaic virus (TMV) in tobacco is conferred by the N gene, which elicits the best characterized plant virus resistance response. Resistance involves multiple responses activated by phytohormones, although those activated by salicylic acid (SA) are the best characterized. At least three responses that inhibit the infection of TMV are activated by SA, through independent pathways: alternative oxidase (AOX), a mitochondrial enzyme involved in regulating reactive oxygen species; pathogenesis related (PR) proteins, synthesized in response to SA via the regulator NPR1; and RNA-dependent RNA polymerase 1, encoded by the gene RDR1. Another pathway, involving the synthesis of an inhibitor of virus replication (IVR), was shown to be independent of SA, as was the pathway involving the transcription factor (TF) ERF5. We found that ERF5 is upstream of and regulates IVR production. By contrast, expression of different PR genes is regulated by the TFs MYB1, WRKY and TGA. The kinetics of gene expression of AOX1, PR1, RDR1 and IVR were examined in tobacco plants by real-time PCR, as were the kinetics of gene expression of ERF5 and MYB1. The plants examined included wild-type NN tobacco, nn tobacco expressing mutants of the N gene that no longer restricted TMV to the inoculated leaf, and NN tobacco silenced for expression of ERF5, MYB1, or both genes. The results indicate complex regulation of expression of the various defense genes, with ERF5 and MYB1 affecting the expression of each other's genes, even though they are in different pathways. A model for the effects of the TFs ERF5 and MYB1 on the expression of the defense effector genes (AOX1, PR1, RDR1 and IVR) will be presented.

SYMPOSIUM 17 – PROTEINS AND POSTRANSLATIONAL REGULATIONS

Sponsored by the Australian Society for Biochemistry and Molecular Biology

SYM-17-01

IMMUNE SIGNALLING BY REDOX-BASED, POST-TRANSLATIONAL PROTEIN MODIFICATIONS

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Post-translational protein modifications add a tremendous amount of complexity to cellular proteomes. The large variety of post-translational modifications and their concurrent appearance in proteins dramatically increase the proteome size from mere thousands to the order of millions of possible protein forms. Most modifications are reversible due to the action of specific enzymes, allowing them to be used as signalling switches that control protein function. While this process is understood relatively well for many modifications, the way in which redox-based modifications are directly controlled remains largely unknown. Cellular redox changes mediate signalling events and responses to the environment in eukaryotic cells. Responses to environmental stress are frequently associated with bursts of reactive oxygen and nitrogen species that can modify cysteine thiols of signalling proteins. Thiol reactivity towards these oxidizing agents leads to formation of, amongst others, disulfides and S-nitrosothiols (SNO), which may alter the function, localization, or activity of signalling proteins that harbour them. Because these modifications occur spontaneously, we questioned how redox-based modifications are employed by the cell as specific, reversible signalling cues. Here we show that the plant immune system utilizes novel and well-established members of the redoxin family, a class of oxidoreductases, to directly engineer the thiol redox states of immune signalling proteins. We will discuss how several redoxins, some unexplored while others well-known, exhibit novel molecular modes of action and may introduce previously unrecognized specificity into redox-based signalling networks that lead to immune gene expression.

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SYM-17-02

INDEPENDENT EVOLUTIONARY RECRUITMENT OF ASPARAGINYL ENDOPEPTIDASE FOR PEPTIDE CYCLISATION

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In all kingdoms of life, head-to-tail ligated and hence ultra-stable peptides may be found. These 'cyclic' peptides have become a focal point for drug designers interested in enhancing the existing bioactivities of these peptides or using the framework to entrench peptides with desirable bioactivities, but on their own would be unstable. To produce cyclic peptides, plants have evolved some extraordinary biosynthetic routes and unusual genetic events that set these biosyntheses in motion. In sunflower seeds, the small cyclic peptide SFTI emerges from within the precursor of an unrelated seed storage napin-type albumin by using asparaginyl endo-peptidase (AEP), a protease known for its role in napin processing, but also one demonstrated to have in vitro trans-peptidation (protein ligation) activity. In this case, SFTI appears to have arisen from a genetic insertion event. In a tropical squash plant called gac, a much larger cyclic peptide was known to exist. Their encoding genes contain a tandem series of cyclic peptides terminating with the more commonly known and potentially ancestral acyclic peptide (a squash, trypsin-inhibitory knottin). Here the cyclic peptide appear to has arisen by an internal genetic expansion event. Expression of both sunflower and gac precursor proteins in Arabidopsis thaliana show the cyclic peptides depend upon asparaginyl endo-peptidase (AEP) for maturation. In the sunflower SFTI precursor protein, AEP was already active on the napin adjacent to SFTI. In the gac precursor protein, AEP appears to have been recruited de novo. Within the plant kingdom there are now three examples AEP-mediated processing of cyclic peptides, each from within unrelated precursor proteins and in phylogenetically distant plant families. This suggests that production of cyclic peptides in angiosperms has evolved in parallel using AEP as a constraining evolutionary channel. This is evolutionary evidence that, in addition to its known roles in proteolysis, under the right conditions AEP is especially suited to performing protein cyclisation in vivo.

SYM-17-03

ANALYSIS OF PROTEIN DOMAINS INVOLVED IN TONOPLAST TARGETING AND FUNCTION OF THE TWO-PORE CHANNEL TPC1

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Two-pore channels (TPCs) constitute a family of endolysosomal cation channels with functions in Ca^{2+} signaling. We used a mutational analysis to investigate the role of channel domains for the trafficking of the Arabidopsis TPC1 to the tonoplast, a process that is generally not well understood in plants. We furthermore identified and characterized protein domains, which are essential for TPC1 activity. The results show that the soluble C-terminus was not essential for targeting but for channel function, while further C-terminal truncations of two or more transmembrane domains impaired protein trafficking. An N-terminal dileucine motif (EDPLI) proved to be critical for tonoplast targeting of AtTPC1, which was independent of the adaptor protein AP-3. Deletion or mutation of this sorting motif, which is conserved among two-pore channels, caused redirection of the protein transport to the plasma membrane. An N-terminal region with a predicted alpha-helical structure was shown to support efficient vacuolar trafficking and was essential for TPC1 function. Similar to their localization in mammalian endosomes and lysosomes, MmTPC1 and MmTPC2 were targeted to small organelles and the membrane of the lytic vacuole, respectively, when expressed in plant cells. Our results on the regulation and trafficking of TPC1 will be discussed.

SYM-17-04

PROTEIN LEVEL REGULATION IN LEAVES

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Recent studies in various organisms have revealed that protein abundance is not only regulated at the transcriptional level, but also during translation and through protein degradation. The mechanisms that link gene expression patterns to protein accumulation are still largely unknown. Studying protein level regulation in leaves we are interested in revealing to what extent protein degradation by the ubiquitin 26S proteasome system (UPS) is involved in the changes of protein levels during day - night changes and in different leaf cell types. Since the UPS is very specific in plants, we are also interested in identifying proteins targeted and regulated by the UPS. In a high-throughput proteomics experiment of leaves after specific inhibition of the proteasome we have gathered a list of about hundred putative UPS target proteins. We obtained new insights into protein homeostasis, as a similar number of proteins decreased after proteasome inhibition. The data suggest that nitrogen assimilation and ammonium cycling are regulated in a day-time dependent manner by the UPS. Further, ribosomal and heat shock proteins accumulate after proteasome inhibition. The results also revealed that reduction of complexity in the full leaf protein extracts is important for identifying more UPS target proteins. This is achieved with cell-type specific extracts of epidermis, mesophyll and vascular tissue and enrichment of ubiquitylated proteins. With the affinity enrichment of poly-ubiquitylated proteins we managed to identify an unprecedented high number of putative UPS target proteins and the cell type specific extracts revealed a high difference between the protein compositions of vasculature, mesophyll and epidermis. Taking these results together will lead to a better understanding of protein level regulation in different leaf cell types and the involvement of the UPS.

SYM-18-01

IDENTIFICATION AND CHARACTERISATION OF THE REGULATORY PATHWAYS FOR ALTERNATIVE OXIDASE IN PLANTS

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While it is well established that AOX responds at transcript, protein and activity levels to a variety of environmental cues, the means by which various signals regulate the expression of AOX is largely unknown, albeit the role of ROS and RNS play an important role(s). Using forward and reverse genetic approaches we have started to dissect the signal transduction pathways that lead to the induction of AOX1a in Arabidopsis thaliana under a variety of treatments. Using these approaches we have not only identified molecular components that regulate the expression of AOX1a, but have started to dissect how the regulatory pathways for AOX1a interact with a variety of other cellular signalling pathways. Specifically we have observed regulators of AOX1a that also appears to be involved in regulating genes that encode chloroplast proteins under stress, antagonistic interaction with hormonal growth promoting pathways have been identified, and uncovered novel stress regulatory pathways in plant cells. An overall working model of the regulation of AOX1a in Arabidopsis will be presented in the context of gaining a understanding of the role of AOX in plant cells under stress and how AOX plays a role in both being responsive to stress, but also integral in defining stress responses in plants.

SYM-18-02

DETERMINANTS BEYOND COMPLEMENTARITY AND EFFICIENT CLEAVAGE ARE REQUIRED FOR STRONG MICROR159 EFFICACY IN *ARABIDOPSIS*

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Plant microRNAs (miRNAs) are critical regulators of gene expression, but despite extensive analyses, uncertainty remains over the principles governing miRNA target recognition and silencing efficacy. Here, we utilize the highly conserved Arabidopsis miR159-MYB33/MYB65 regulatory module to explore these principles. Firstly, we show perfect central complementarity is not required for strong silencing. Artificial miR159 variants with two cleavage site mismatches can potently silence MYB33/MYB65, fully complementing a loss-of-function mir159 mutant. Moreover, these miR159 variants can cleave MYB33/MYB65 mRNA, although cleavage appears attenuated, as MYB33/MYB65 mRNA levels increase with increasing cleavage site mismatches. Nonetheless, high levels of un-cleaved MYB33/MYB65 transcripts are strongly repressed by a non-cleavage mechanism. Finally, we identify highly conserved nucleotides that flank the miR159 binding site in MYB33 and demonstrate they are critical for efficient silencing, implying that miRNA target recognition extends beyond the miRNA binding site. This highlights that the context in which the miRNA binding site resides is a key determinant in controlling miRNA efficacy. We are currently trying to define what these contextual factors are.

SYM-18-03

REGULATION OF FLOWERING BY TREHALOSE-6-PHOSPHATE SIGNALING

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The induction of flowering is a central event in the life cycle of plants. When timed correctly, it helps ensure reproductive success, and therefore has adaptive value. Because of its importance, flowering is under the control of a complex genetic circuitry that integrates environmental and endogenous signals. Trehalose-6-phosphate (T6P) has been shown to act as signaling molecule in coordinating carbohydrate status with diverse developmental processes. Interestingly, Arabidopsis thaliana plants deficient in the key T6P-synthesizing enzyme TREHALOSE-6-PHOSPHATE SYNTHASE 1 (TPS1) are extremely late flowering. We show that T6P regulates expression of several flowering-time related genes throughout the plant. In the leaf vasculature T6P is absolutely required for expression of FLOWERING LOCUS T (FT). In addition, TPS1/T6P regulates the expression of miR156 and some of its target genes of the SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPL) family of transcription factors at the shoot apex. We hypothesize that the TPS1/T6P pathway provides a way for plants to integrate an environmental signal, the activation of FT in response to increasing day length in spring, with a physiologic signal, the presence of high carbohydrate levels, as indicated by T6P. Together these two inputs ensure that FT is only expressed when the conditions are optimal, that is, day length exceeds a certain minimum and the carbohydrate state of the plant supports the energy-demanding transition to flowering and seed production. In addition, TPS1/T6P modulates flowering through regulation of the miR156/SPL module, which in wild type plants ensures that flowering will eventually occur even in the absence of inductive environmental cues.

SYM-18-04

INTERPLAY BETWEEN THE SHOOT MERISTEM AND LATERAL ORGAN BOUNDARY IS ESSENTIAL FOR REGULATING SHOOT ARCHITECTURE

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Shoot architecture is regulated by the maintenance, activity and identity of the shoot meristem. Genetic studies demonstrate that homeodomain transcription factors SHOOT MERISTEMLESS (STM), PENNYWISE (PNY) and POUND-FOOLISH (PNF) play a fundamental role in specifying shoot meristem cell fate in Arabidopsis. In addition, STM, PNY and PNF also regulate inflorescence architecture, as a decrease in the activity of these homeodomain transcription factors perturbs internode patterning, axillary meristem specification and organ separation. Genetic studies show that the lateral organ boundary also controls shoot architecture by regulating meristem maintenance, organ separation as well as axillary meristem formation. The goal of this study is to investigate how the interplay between the shoot meristem and lateral organ boundary functions to regulate shoot architecture during inflorescence development in Arabidopsis. Experimental results suggest that STM-PNY/PNF transcriptional complexes act to directly repress boundary identity in the shoot meristem. However, STM alone or in combination with another homeodomain transcription factor functions to directly activate boundary identity on the flanks of the shoot meristem. The direct regulation of lateral organ boundary identity by these homeodomain transcription factors is essential for regulating stem cell homeostasis, axillary meristem formation and internode patterning as well as flower meristem identity.

ICAR 2013 Workshop Program

24th International Conference on Arabidopsis Research

	TIME	ROOM	CHAIRS	
TUESDAY 25th June 2013				
WORKSHOP **1**	12:45 - 14:15	Parkside Auditorium	E. Dennis, J. Peacock, D. Wagner	**EPIC: Epigenomes of Plants International Consortium**
WORKSHOP **2**	12:45 - 14:15	110B	C. Foyer, J Whelan, M. Considine	**Redox signaling in mitochondria**
WEDNESDAY 26th June 2013				
WORKSHOP **3**	12:45 - 14:15	Parkside Auditorium	R. Simon, R. Sozzani, S. de Vries	**Live Imaging of Protein Functions**
WORKSHOP **4**	12:45 - 14:15	110B	C. Town, B. Meyers, M. Nordborg	**International Arabidopsis Informatics Consortium: The transition from TAIR to the Arabidopsis Information Portal (AIP)**
THURSDAY 27th June 2013				
WORKSHOP **5**	12:45 - 14:15	Parkside Auditorium	B. Meyers, N. Imin, M. Djordjevic	**The Small Regulatory Molecules: microRNAs and peptides**
WORKSHOP **6**	12:45 - 14:15	110B	M. Nowak, I. Hara-Nishimura, F. Van Breusegem	**Programmed Cell Death during Arabidopsis Development and Stress Response**
FRIDAY 28th June 2013				
WORKSHOP **7**	13:45 - 15:15	110A	X. Sirault, J. Borevitz, A. Weirmann	**Genetic Traits from Phenomics Data**
WORKSHOP **8**	13:45 - 15:15	110B	M. Williams, G. Estavillo	**Teaching workshop for early career scientists**
COFFEE BREAK	15:15 - 15:45			
WORKSHOP **9**	15:45 - 17:15	110A	R. Jost, H. Takahashi, L. Nussaume	**Plant nutrition in the face of impending global resource limitation - opportunities for model plant research**
WORKSHOP **10**	15:45 - 17:15	110B	A. Jones, H. Millar, J. Heazlewood	**Using proteomics to identify receptor complexes and signalling events**

WORKSHOPS

WORK-01-01

CHROMATIN REMODELLING IN INDUCIBLE GENE EXPRESSION

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The NSF funded Epigenomics International Consortium (EPIC) Research Collaborative Network Initiative aims to facilitate and coordinate elucidation of plant epigenomes. The Initiative currently has a 30 member international planning committee. A position paper about the goals of EPIC was published in The Plant Cell in 2012 (http://www.plantcell.org/content/early/2012/06/28/tpc.112.100636) and the activities of the initiative are showcased on the EPIC website (https://www.plant-epigenome.org/). I will briefly summarize recent advances of EPIC. In addition I will discuss work from my own lab, which focuses on the chromatin state of the nucleus as a critical determinant of cell identity and of appropriate responses to environmental cues. One central mechanism for altering the chromatin state is chromatin remodeling, a process that uses the energy derived from ATP hydrolysis to change the interaction between the genomic DNA and the histone octamer in the nucleosome. SWI/SNF ATPases are among the best-studied chromatin remodelers. My lab's investigations have focused on the roles, mechanism of action, and regulation of SWI/SNF ATPases in plants. In Arabidopsis, there are 3 classes of SWI/SNF ATPases: SPLAYED (SYD), BRAHMA (BRM) and MINUSCULE (MINU). Like their metazoan counterparts, Arabidopsis SWI/SNF ATPases control both pluripotency and differentiation. In addition, they have key roles in biotic and abiotic stress responses. Recently, we have focused our studies on elucidating what controls the specificity of the activity of the SWI/SNF ATPases. We have identified families of transcription factors that preferentially recruit SWI/SNF chromatin remodelers to genomic target loci and post-translational modifications that modulate SWI/SNF ATPase activity.

WORK-01-02

THE EPIC-COGE BROWSER FOR *ARABIDOPSIS* EPIGENOMIC DATA

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Epigenetic regulatory pathways control mRNA levels both transcriptionally and posttranscriptionally, and pioneering work in Arabidopsis thaliana has helped define these processes. For this reason, there is a wealth of epigenomic information already available for this model plant. However, it is almost entirely unusable to the wider research community due to the computational intensive procedures needed to leverage these data resources. For this reason, we have begun to develop an easy to use web-based system to store, access, and visualize Arabidopsis epigenetic data in a comparative genomics context: the EPIC-CoGe Browser. To do this, we are building extensions to CoGe that will 1) implement a high-performance and portable data engine that can store thousands of plant epigenetic experimental datasets; 2) enable researchers to load their own experiments, keep them private, and share them will collaborators; 3) develop a web-based visualization system for overlaying and partitioning epigenetics data onto genomic annotations; 4) enable researchers to select and organize sets of epigenetics experiments for dynamic visualization; and 5) load all publicly available epigenetics datasets for Arabidopsis. Here, the progress that has been made towards these aims will be described. Additionally, an overview of features that are now available to the epigenetics research community will be provided. Specifically, we have already made significant progress towards all of these goals, which are currently available for public use. Many of these functionalities and features will be described and displayed. Finally, any and all feedback and comments from the epigenetics community are welcomed and appreciated.

WORK-01-03

AN ENDOGENOUS MOBILE RNAI PATHWAY REQUIRED FOR STRESS RESPONSE IN PLANTS

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Small RNAs in plants play essential roles in development, stress-responses and protection of the genome from invading viruses and transposons. The bulk of research has focused on two major classes of small RNA; miRNAs and transposon and repeat-derived siRNAs. The Arabidopsis genome encodes potentially hundreds of inverted-repeats (IR's) which, upon processing, produce huge amounts of siRNAs in a manner almost identical to that of exogenous IR (or RNAi) transgenes. Here, we focus on one such IR, termed IR71. We demonstrate that IR71-derived siRNAs are expressed in an accession-specific manner, are induced in response to several types of abiotic stress and are mobile between several different tissues. Utilising a T-DNA insertion that specifically eliminates IR71 siRNAs, we demonstrate that the mutant is more susceptible to heat stress, as opposed to normal conditions where the mutant is comparable to wild type. Transcriptome analysis, in combination with small RNA sequencing, indicates that unique species of siRNA are produced under stress conditions, which in turn target several genes known to be involved in integrating stress responses. Using the same approach, we demonstrate that the mobile IR71 siRNAs also target endogenous transcripts. Collectively, these results demonstrate the existence of a bona fide endogenous mobile RNAi pathway which is likely to play a key role in perceiving and integrating multiple stress response pathways.

WORK-01-04

EPIGENETIC REGULATION OF CAROTENOID BIOSYNTHESIS: IMPACTS ON PLANT DEVELOPMENT

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In plants, carotenoids are required for photosynthesis, photoprotection and the biosynthesis of at least two hormones, namely abscisic acid and strigolactones. After a decade of advances in understanding the biosynthetic enzymes, the next frontier is to discover what regulates carotenoid biosynthesis, accumulation and storage. The carotenoid biosynthetic pathway bifurcates after lycopene to produce lutein or beta-carotenes and its derivatives. Thus the branch point modulates which carotenoids accumulate [Cazzonelli, 2011 Functional Plant Biology]. We have shown how the branch point can be regulated by a chromatin-modifying histone methyltransferase, SET DOMAIN GROUP 8, (SDG8), targeting the carotenoid isomerase (CRTISO) [Cazzonelli et al., 2009 Plant Cell]. SDG8 controls the permissive expression of a small number of genes by histone methylation of lysine 4 and/or 36 of chromatin surrounding key gene targets such as CRTISO [Cazzonelli et al., 2009 Plant Signaling & Behavior]. Regions within the CRTISO promoter are required for SDG8 recruitment as well as function, and tissue specific expression of CRTISO is similar to that of SDG8 [Cazzonelli et al., 2010 Molecular Plant]. We are exploring the molecular nature by which SDG8 regulates CRTISO and the production of carotenoid-derived signaling molecules. This presentation will consider why an epigenetic regulator of flowering and shoot branching, a histone methyltransferase, would also regulate carotenoid biosynthesis.

WORK-01-05

RECRUITMENT AND CHANGES TO HISTONE MODIFICATIONS ON *FLC* CHROMATIN IN RESPONSE TO CHANGES IN TRANSCRIPTION

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The FLC gene encodes a MADS box repressor of flowering in Arabidopsis which is stably repressed by vernalization (extended cold) in a process that involves the addition of the repressive histone modification H3K27me3 (Histone H3 lysine 27 trimethylation) by polycomb repressive complex 2 (PRC2). We have previously shown that an FLC transgene placed under the control of a dexamethasone-inducible promoter is able to recruit H3K27me3 when it is not being transcribed. We also showed that that H3K27me3 changes in response to changes in transcription, with H3K27me3 being removed when transcription is switched on and added when transcription is switched off (Buzas et al, Plant J 65:872), with changes in H3K27me3 being tightly linked to changes in transcription (Anderssen and Helliwell, J Math Biol 2012). These results suggested that the increased H3K27me3 following vernalization could be explained as being a consequence of cold stopping transcription of a gene (FLC) that has the intrinsic property of recruiting H3K27me3 when not transcribed. We have investigated the properties of this FLC transgene further. The active marks H3K4me3 and H3K36me3 are present on the transgene when it is transcribed; changes in these marks are very tightly linked to the transcription rate of the transgene. We have further investigated the H3K27me3-recruiting properties of the FLC transgene by dissection of the region. The results of this analysis show that all regions tested can recruit H3K27me3, suggesting that the FLC gene contains multiple polycomb recruiting sequences.

WORK-01-06

IDENTIFICATION OF LONG NON-CODING RNAS INVOLVED IN RNA-DIRECTED DNA METHYLATION IN PLANTS

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RNA-directed DNA methylation (RdDM) plays a fundamental role in gene regulation and plant defence against invasive DNA. RdDM is induced by 24-nt small interfering RNAs (siRNAs) which are loaded onto Argonaute 4 (AGO4) to form an effector complex. This effector complex is recruited to genomic loci through physical interaction with long non-coding RNAs (lncRNAs), which function as a scaffold to determine the exact target genomic region at which a DNA methylation enzyme catalyses cytosine methylation resulting in de novo DNA methylation and gene repression. While 24-nt siRNAs have been well identified, lncRNAs have not been well characterised which has hindered the identification of RdDM-regulated genes in plants. Using nuclear RNA immunoprecipitation and Illumina sequencing, we constructed a highly enriched library and obtained sequences of ncRNAs specifically associated with AGO4. Comparison of AGO4-associated ncRNAs with microarray expression data of RdDM mutants identified novel protein coding gene targets of RdDM. Surprisingly, a large proportion of these potential RdDM target genes were down-regulated in RdDM mutants suggesting that they are normally activated by RdDM. These RdDM-activated genes are more enriched than the RdDM-repressed genes for AGO4-associated ncRNAs derived from the gene body. Thus, in addition to its canonical function in gene repression, RdDM may play a role in maintaining or activating gene expression, possibly by directing gene body methylation. Functional classification of these RdDM-activated genes show an over-representation of stress-responsive genes, many of which are induced upon infection by the fungus, Fusarium oxysporum. We propose that the RdDM pathway functions to maintain the expression of stress response genes to confer disease resistance. Grant acknowledgement: Australian Research Council Future Fellowship (FT0991956).

WORK-02-01

TISSUE SPECIFICITY OF PROTEINS OF THE MITOCHONDRIAL TCA CYCLE REVEAL BY SELECTED REACTION MONITORING MASS SPECTROMETRY

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Studies in Arabidopsis of sub cellular biochemical pathways typically isolate organelles by grinding cell cultures, shoot cultures or whole plants. However recently the diversity of the TCA cycle in different tissues has been revealed at the metabolite level using metabolic network models and labelling studies. Here we have examined the diversity of all 37 proteins of the 9 enzymes that make up the TCA by quantifying their abundance in mitochondria isolated from Arabidopsis leaves, cell culture, flowers, roots, stems and siliques using a selected reaction monitoring mass spectrometry approach. It has revealed that the components of the TCA cycle are generally most abundant in leaf tissue as would be expected, however in root tissue aconitase and succinate dehydrogenase were more abundant than in leaves. In cell culture tissue a large increase in the abundance of citrate synthase was observed compared with leaf tissue and when comparing siliques with stems it revealed a greater abundance of citrate synthase and succinate dehydrogenase and a decrease in the abundance of 2-oxoglutarate dehydrogense. Together these results show that the TCA cycle and likely many other major biochemical pathways are very dynamic and the abundance of their components varies depending on the demands of these tissue in which they are housed.

WORK-02-02

DYNAMICS BEHIND THE STATIC MITOCHONDRIAL PROTEOME THROUGH PROTEIN TURNOVER ANALYSIS IN ARABIDOPSIS

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Plant mitochondria have many components that rarely change in abundance between tissue types or with treatments. As shotgun approaches in proteome studies only look at changes in protein abundance, little information on what is occurring to maintain this static proteome can be obtained. To explore what these steady-states mean we need to be able to analyse protein synthesis and degradation rates. We have done this using progressive stable isotope labelling to give a new window on the control of protein abundance in mitochondria as we seek to determine the relative age of the proteins that we see. Through progressive ^{15}N labelling of plant cells from nitrate and ammonia salts, coupled to modelling incorporation fits, we can calculate the rate at which proteins which are static in abundance in the proteome are turning over. Through combining this with separation of protein complexes and subcomplexes by native electrophoresis, we can observe the in vivo turnover rate of assembly intermediates of protein complexes. Through this we have gained new insights into the assembly and the in vivo subcomplexes of Complex I and Complex V. We are now also gaining new information on the subsets of soluble mitochondrial proteins that rapidly turnover to control respiratory biogenesis and function in Arabidopsis mitochondria.

WORK-02-03

THE ROLE OF AUXIN IN THE MITOCHONDRIAL STRESS RESPONSE

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Plants must deal effectively with unfavourable growth conditions that necessitate a coordinated response to integrate cellular signals with mitochondrial retrograde signals. On the other hand, regulation of plant growth and development by genetic and environmental signals relies on tightly controlled spatial and temporal distribution of plant hormones. A genetic screen was carried out to identify regulators of alternative oxidase (rao mutants) using AOX1a expression as a model system to study retrograde signalling in plants. Two mutants, named rao3/big and rao4/as1 exhibiting altered polar transport and differential distribution of the plant hormone auxin had an exaggerated response to antimycin A (an inhibitor of mitochondrial electron transport chain). In our study we examined further the role of auxin in mediating mitochondrial stress response.

WORK-02-04

KNOCKDOWN OF MITOCHONDRIAL-LOCATED GLUTAREDOXIN S15 REVEALS A ROLE IN ARSENIC TOXICITY

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Glutaredoxins (Grx) are small ubiquitous enzymes, generally involved in re-reduction of diverse oxidative modifications using glutathione. In recent years data has accumulated about the important role Grxs play in the cellular redox network, regulating the activity of key enzymes in the Calvin cycle and TCA cycle, while during stress situations they can regenerate antioxidant enzymes such as peroxiredoxins or low molecular weight antioxidants such as dehydroascorbate. In order to gain a better understanding of how energy metabolism and redox regulation mediated by Grxs are linked, mitochondria are of specific interest. Despite evidence for multiple Grxs in Arabidopsis mitochondria, combined analysis of subcellular localisation of GFP fusion proteins and proteomics of isolated mitochondria has shown that there is really only one key player, GrxS15. In order to uncover the impact of GrxS15 in Arabidopsis mitochondria different in vitro and in vivo experimental approaches were employed. A GrxS15 T-DNA insertion line as well as an RNAi line show altered expression patterns and subsequently lead to a knockdown at the protein level. The reduction of GrxS15 amount results in a growth phenotype with significantly shorter roots compared to wildtype plants. This phenotype renders plants more susceptible to arsenic poisoning. Different forms of arsenic promote the generation of an even stronger root growth phenotype and the change of the overall root system development. Furthermore, the transgenic lines show smaller rosettes compared to wildtype plants. These results indicate that mitochondria are involved in the detoxification of arsenic compounds, and moreover reveal that GrxS15 is a crucial element within this pathway. Taken together these findings have greatly improved the current understanding of Grx function in plant mitochondria.

WORK-03-01

THE INTERNATIONAL ARABIDOPSIS INFORMATICS CONSORTIUM: HOW WE GOT HERE, AND WHAT'S NEXT FOR ARABIDOPSIS INFORMATICS

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The International Arabidopsis Informatics Consortium (IAIC) was initiated in 2010 to address increasing bioinformatics needs for Arabidopsis data and in response to funding concerns for the community's primary database, TAIR. The goal of this community-led international initiative is to manage the increasing amounts and types of data and to leverage growing resources, knowledge, and collaborations. The Arabidopsis Information Portal (AIP) is the informatics infrastructure of the IAIC, which will provide the framework within which the Arabidopsis genome sequence data will be managed and accessed, along with a diverse array of resources that currently exist, and more that will be developed in the future. Once the AIP is established and accessible by the community, additional modules can be linked in, allowing data integration. The IAIC will connect members of the community to interact, coordinate, and develop resources across the globe. This talk will briefly outline the steps the community has taken to get to this point in IAIC development, and it will introduce the next steps for AIP development. It will provide the background for the remaining speakers who will discuss key parts of the consortium including the current status of the TAIR:iPlant transition, the current vision for the AIP, the Scientific Advisory Board overseeing our progress, one of the core Arabidopsis stock centers and its linkage to the IAIC, and examples of several research projects that are expected to be integrated within the AIP as community-initiated 'modules'.

WORK-03-02

AIP: PHYSICAL RESOURCES - IN SEARCH OF THE MISSING LINK(S)

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AIP does not make provision for a Germplasm module; it explicitly expects this crucial central physical resource and associated access to develop externally from expertise at the Stock Centres. AIP also expressly prefers that module data be transmitted in meta-data rich ontology driven formats derived from local expertise and pre-processing. Current NASC Web Services (120+ WS) are SOAP2 (and legacy BioMOBY) with initial RESTful conversions already under specification. We wish to expand to both alternative WS approaches given past experience with the strength of user-choice drivers that exist in the bioinformatics community. Although SOAP is an obvious standard, REST has been adopted by mainstream Web 2.0 service providers including Yahoo, Google, and Facebook - who have deprecated or passed on SOAP and WSDL-based interfaces in favour of an arguably easier-to-use, resource-oriented model to expose their services. For example, ThaleMine delivers data by JSON via REST-ful service, and iPlant has its REST API Agave Data service. We need to make sure that we support the approaches that our community are likely to require especially those that will be incorporated into the AIP model.

WORK-03-03

THE EPIC-COGE BROWSER FOR *ARABIDOPSIS* EPIGENOMIC DATA

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Epigenetic regulatory pathways control mRNA levels both transcriptionally and posttranscriptionally, and pioneering work in Arabidopsis thaliana has helped define these processes. For this reason, there is a wealth of epigenomic information already available for this model plant. However, it is almost entirely unusable to the wider research community due to the computational intensive procedures needed to leverage these data resources. For this reason, we have begun to develop an easy to use web-based system to store, access, and visualize Arabidopsis epigenetic data in a comparative genomics context: the EPIC-CoGe Browser. To do this, we are building extensions to CoGe that will 1) implement a high-performance and portable data engine that can store thousands of plant epigenetic experimental datasets; 2) enable researchers to load their own experiments, keep them private, and share them will collaborators; 3) develop a web-based visualization system for overlaying and partitioning epigenetics data onto genomic annotations; 4) enable researchers to select and organize sets of epigenetics experiments for dynamic visualization; and 5) load all publicly available epigenetics datasets for Arabidopsis. Here, the progress that has been made towards these aims will be described. Additionally, an overview of features that are now available to the epigenetics research community will be provided. Specifically, we have already made significant progress towards all of these goals, which are currently available for public use. Many of these functionalities and features will be described and displayed. Finally, any and all feedback and comments from the epigenetics community are welcomed and appreciated.

WORK-03-04

POSMED: ANOTHER GATEWAY TO THE AIP DATABASES FROM LITERATURE

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Genome sequence data and publications are two of the most heavily relied-upon information sources for many biologists. Gene identifiers assigned to genomic regions play a hub role linking various information resources; however, so far very few publications are linked to gene identifiers. Although dedicated teams manually curate publications about genes, the thousands of articles published every day make it difficult for manual curation to timely integrate the latest publications without an automated text-mining system mapping publications to database identifiers. It is imperative for the AIP to systematically integrate gene data directly with the biological literature, so that users can easily access sequence-based gene information from conventional literature search engines. To help overcome the lack of integration between genomic information and biomedical literature, we developed Positional MEDLINE (PosMed) that maps publications onto genomic positions automatically (http://biolod.org/PosMed) and provides a powerful search of genes from literature. Given a user-specified query, PosMed rabidly performs a full-text search of each document in MEDLINE and then ranks gene identifiers mapped to hit documents in order of statistical significance of associations between hit document and each gene, so that users can quickly access the most relevant genes related to the hit documents. We are willing to provide the PosMed search engine to the AIP community, and want to find out what kinds of associations between genes and publications need to be established for the community to investigate plant physiology more efficiently. Although automated text-mining could include a certain amount of false positives and false negatives in gene-to-publication matching results, using two gateways from both automated rapid curation and slow but careful manual curation to AIP databases from literature should, we believe, compensate each other well.

WORK-03-05

SUBCELLULAR REACTION ROOM PROTEOMES FOR RECONSTRUCTING A COMPARTMENTALIZED MODEL OF ARABIDOPSIS METABOLISM

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In the post genome-era, omics data are widely available and current challenges lie in developing adequate computational pipelines for their integration and analysis. Genome, proteome and other omics data can be integrated into genome-scale metabolic models. Such models have been used to describe metabolism in microorganisms and first computational models also contribute to plant metabolic engineering to target molecular breeding. Yet accurate computational modelling of plant metabolism is hampered by incomplete knowledge about protein function, pathway membership and subcellular localization. The plant proteome is highly compartmentalized and subcellular protein location significantly affects model characteristics. The subcellular location database for Arabidopsis proteins (SUBA3, http://suba.plantenergy.uwa.edu.au) combines manual literature curation of large-scale subcellular proteomics, fluorescent protein visualization and protein-protein interaction datasets with subcellular targeting calls from 22 prediction programs. To determine protein location as objectively as possible, we have developed a Bayesian approach that incorporates experimental localization and targeting prediction data to best estimate subcellular protein location. These data have been used to construct genome-scale metabolic models for Arabidopsis cells compartmentalized into six organelle locations. We have expanded our localization data to include experimental and predicted sub-organellar locations. These data are used to generate SUBcellular Arabidopsis reaction room proteomes (SUBArr) and their connection proteomes. Such reaction room data is currently used for reconstructing compartmentalized metabolic models of the peroxisome, plastid and mitochondrion within a connecting framework. The SUBArr pipeline will create an expanding tool for modelling plant responses to nutrients availability and genomic perturbations.

WORK-03-06

MINING POST TRANSLATIONAL MODIFICATIONS IN ARABIDOPSIS USING THE MODHUNTER

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The past decade has seen the development of a plethora of online proteomics resources in Arabidopsis reflecting multiple large-scale studies. These resources exist independently and lack a level of integration. The Multinational Arabidopsis Steering Committee, Proteomics (MASCP) has addressed this issue through the development of a proteomics aggregation portal, MASCP Gator (http://gator.masc-proteomics.org/). The portal provides a summary of proteomics and protein information aggregated directly from ten online resources. The development of this portal has enabled us to develop a bioinformatics technique to identify likely regions of post-translational modifications in proteins of Arabidopsis. The ability to locate and identify post translational modifications experimentally by mass spectrometry is extremely challenging and there is a requirement for complementary techniques. Virtually all large-scale proteomics analyses in Arabidopsis have identified proteins with unmodified peptides. Collectively, these data reveal modified regions of a protein as unmatched areas within a protein model. Using a recent large-scale N-linked glycosylation survey as a test set, we could demonstrate that unmatched regions represent modification hotspots in proteins. These sites can be further targeted for investigation and characterization. We have now developed a method to locate putative regions with modifications by exploiting mass spectral data in the public domain and are attempting to develop this into a functional portal for the assessment modifications in proteomic datasets.

WORK-04-01

DYNAMICS GAINED FROM FLUORESCENT PROTEIN TECHNOLOGIES

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Stem cells are the building blocks for different cell types and tissues in all multicellular organisms. Overall growth rate and biomass are largely regulated by the temporal and spatial control of stem cell regeneration and differentiation of their progeny. When a stem cell divides it produces both a copy of itself and a daughter cell that can develop into different cell types. Understanding how stem cells are maintained and organized should provide insight into how multicellular organisms initiate and maintain growth of their tissues and organs. There are several excellent models for studying these processes in animals and plants. The Arabidopsis root, due to the continuous post-embryonic nature of its development, and the presence of a confined stem cell niche, has emerged as a leading system to address these questions. A key to system-level understanding of stem cell maintenance is the ability to analyze the dynamics of networks in the context of a living organism. The development of quantitative models to describe these dynamics, as well as parameter estimation to improve existing models, depends on the ability to obtain quantitative information about various proteins that are part of the regulatory network. Recent developments in the field of imaging have provided the tools to enable the observation of network dynamics in living organisms. Therefore, to measure molecular dynamics and concentrations we took advantage of newly developed fluorescence correlation spectroscopy techniques based on confocal laser scanning microscopy imaging of Arabidopsis roots. These approaches enable the quantification of protein mobility, concentration and binding with high spatial resolution. The integration of imaging tools with genome-wide approaches and the modeling of the regulatory networks offer the unique advantage of monitoring the function of biological circuits over time at cellular resolution.

WORK-04-02

A STEP TOWARD UNDERSTANDING SPATIOTEMPORAL DYNAMICS OF NETWORKS REGULATING PROTEIN MOVEMENT AND ASYMMETRIC CELL DIVISION IN THE ARABIDOPSIS ROOT MERISTEM

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In the Arabidopsis root meristem, patterning is controlled by key proteins that act as molecular switches for cell fate determination. The cell fate determinant SHORTROOT (SHR) has been shown to move between layers in the root meristem. SHR binds and activates its target and binding partner SCARECROW (SCR) which promotes periclinal cell division in the cortex/endodermis stem cells (Cui et al., 2007, Helariutta et al., 1996). SHR movement is determinant for the spatial location of the periclinal cell division. Here we show that SHR movement is regulated by JACKDAW and other 'Bird' members of a sub-clade of Zinc-finger nuclear proteins which confine SHR to the nucleus and restrict its to the endodermis. Mutations in a subset of Bird genes lead to SHR spread and loss of tissue boundaries. In addition, protein interaction studies in living roots suggest a spatiotemporal protein complex dynamics between Bird proteins and SCR/SHR. We also show that divisions and acquisition of endodermal cell fate require combinatorial activity of SHR targets.

WORK-04-03

MODERATION OF ARABIDOPSIS ROOT STEMNESS BY CLAVATA1 AND ARABIDOPSIS CRINKLY4 RECEPTOR KINASE COMPLEXES

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The root system of higher plants originates from the activity of a root meristem, which comprises a group of highly specialized and long-lasting stem cells. Their maintenance and number is controlled by the quiescent center (QC) cells and by feedback signaling from differentiated cells. Root meristems may have evolved from structurally distinct shoot meristems; however, no common player acting in stemness control has been found so far. We show that CLAVATA1 (CLV1), a key receptor kinase in shoot stemness maintenance, performs a similar but distinct role in root meristems. We report that CLV1 is signaling, activated by the peptide ligand CLAVATA3/EMBRYO SURROUNDING REGION40 (CLE40), together with the receptor kinase ARABIDOPSIS CRINKLY4 (ACR4) to restrict root stemness. Both CLV1 and ACR4 overlap in their expression domains in the distal root meristem and localize to the plasma membrane (PM) and plasmodesmata (PDs), where ACR4 preferentially accumulates. Using multiparameter fluorescence image spectroscopy (MFIS), we show that CLV1 and ACR4 can form homo- and heteromeric complexes that differ in their composition depending on their subcellular localization. We hypothesize that these homo- and heteromeric complexes may differentially regulate distal root meristem maintenance. We conclude that essential components of the ancestral shoot stemness regulatory system also act in the root and that the specific interaction of CLV1 with ACR4 serves to moderate and control stemness homeostasis in the root meristem. The structural differences between these two meristem types may have necessitated this recruitment of ACR4 for signaling by CLV1.

WORK-04-04

VISUALIZING BRI1-SERK3 HETERO-OLIGOMERS IN *ARABIDOPSIS* ROOTS

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Brassinosteroids (BRs) are plant hormones that are perceived by plasma membrane (PM)-located receptors such as Brassinosteroid Insensitive 1 (BRI1) in Arabidopsis thaliana. Additionally, BR signalling is also dependent on the function of the Somatic Embryogenesis Receptor-like Kinases (SERK) co-receptor family. Transgenic plant lines expressing BRI1-GFP and SERK3-mCherry were generated and analyzed by confocal microscopy and FRET-FLIM to visualize the molecular events upon initiation of BR signaling. In accord with the current model of BR signal transduction, a time-dependent and ligand-induced hetero-oligomerization between BRI1 and SERK3 was observed, similar to previous reports using coimmunoprecipitation. In addition, the spatially resolved FLIM images enabled us to localize these BRI1-SERK3 receptor complexes to restricted areas within the PM of live epidermal root cells, a cell file known to exhibit active BR signaling. In contrast to the established BRI1 signaling model, FRET-FLIM revealed that a substantial amount of the BRI1-SERK3 hetero-oligomers was preformed.

WORK-04-05

DESIGN AND USE OF FLUORESCENT BIOSENSORS IN PLANTS

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The need for tools that allow us to quantify the concentrations and dynmaics of ion and metabolites, as ell as biophysical processes such as voltage an tension have sparked the engineering of a wide range of genetically encoded fluorescent sensors. We will discuss the engineering of both single fluorophore and FRET-based sensors, their in vitro characterization, their implementation in intact plants, the tools and technologies for live imaging, the potential pitfalls as well as platforms that support imaging such as the RootChip. We will also discuss the use of such sensors for high throughput screening and gene discovery. Jones A., Grossmann G., Danielson, J.A.H., Sosso D., Chen L. Q., Ho, C.H. & Frommer W.B. (2013) In vivo biochemistry: Applications for small molecule biosensors in plant biology. Curr. Opin. Plant Biol. Apr 12. doi:pii: S1369-5266(13)00030-7. 10.1016/j.pbi.2013.02.010. [Epub ahead of print] Bermejo C., Haerizadeh F., Sadoine M., Chermak D. & Frommer W.B. (2013) Differential regulation of glucose transport activity in yeast by specific cAMP signatures. Biochem. J. Mar 15. [Epub ahead of print] San Marten A., Gutierrez R., Ceballo S., Ruminot I., Lerchundi R., Baeza-Lehnert F., Frommer W.B., & Barros F.L. (2013) A genetically-encoded FRET lactate biosensor shows that neurons are energized by lactate. PLoS One 8(2), e57712. Grossmann G., Meier M., Cartwright H.N., Sosso D., Quake S.R., Ehrhardt D.W. & Frommer W.B. (2012) Time-lapse fluorescence imaging of Arabidopsis root growth with rapid manipulation of the root environment using the RootChip. J Vis Exp. 65, 4290. Okumoto S., Jones A. & Frommer W.B. (2012) Quantitative imaging with fluorescent biosensors. Ann. Rev. Plant Biol. 63, 663-706. Chen L.Q., Qu X.Q., Hou B.H., Osorio S., Fernie A.R. & Frommer W.B. (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science 335, 207-211.

WORK-04-06

ROOTS ON CHIPS - MICROFLUIDIC DEVICES FOR IMAGING OF PLANT ROOTS

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Time lapse microscopy of growing roots enables the observation of root development, the measurement of metabolite fluxes, and the study of protein dynamics. To observe living roots over longer time periods and under controlled conditions, we need to cultivate plants directly at the microscope with the possibility to precisely control the root environment or to apply chemical treatments. Microfluidic perfusion platforms for plants allow for integration of live-cell imaging of growth and metabolism with rapid modulation of the microenvironment. In devices like the RootChip, multiple roots can be stimulated and imaged in parallel and the experimental setup can be automated. By expressing genetically encoded fluorescence sensors, we are able to follow the flux of metabolites in growing roots over several days at sub-cellular resolution.

WORK-04-07

4D LIGHT SHEET BASED IMAGING REVEAL THAT SHAPE OF PLANT LATERAL ROOT IS DEPENDENT ON THE PROPERTIES OF THE OVERLAYING TISSUES RATHER THAN CELL DIVISION PATTERN

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In plants, the root system is responsible for the uptake of all nutrients and water the plants needs to sustain viability and growth. In Arabidopsis thaliana, it consists of an embryo-derived primary root, from which a variable number of lateral roots branches. The lateral root primordia (LRP) originate from pericycle cells located deep within the parental root and have to emerge through several cell layers of the main root. These overlaying tissues place biomechanical constraints on the LRP that are likely to impact its morphogenesis. We examined using light-sheet based fluorescence microscopy on live samples the interplay between the patterns of cell division, organ shape and overlaying tissues on LRP morphogenesis. 3D/4D image analysis revealed that early stage LRP exhibit tangential divisions that create a ring of cells corralling a population of rapidly dividing cells at its centre. The patterns of division in the latter population of cells during LRP morphogenesis are not stereotypical. In contrast, statistical analysis demonstrated that the shape of new LRP is highly conserved. We tested the relative importance of cell division pattern versus overlaying tissues on LRP morphogenesis using mutant and transgenic approaches. The mutant aur1 aur2 disrupts the pattern of LRP cell divisions yet does not impact the evolution of organ shape. In contrast, manipulating the properties of overlaying tissues disrupted LRP morphogenesis. We conclude that the interaction with overlaying tissues, rather than the precise pattern of divisions, is most important for LRP morphogenesis and optimizes the process of lateral root emergence.

WORK-05-01

miRNA EVOLUTION IN THE *CAMELINEAE*

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MicroRNAs (miRNAs) are short RNA sequences involved in gene regulation through translational inhibition and transcript cleavage that are found in both plants and animals. The miRNAs are processed from imperfect foldback structures and incorporated into RNA-induced silencing complexes before targeting transcripts with high sequence complementarity. Some miRNAs are evolutionarily deeply rooted and their homology with their targets is maintained through purifying selection. Only a few lineage-specific miRNAs have been studied for evolutionary constraints. The increasing number of related high-quality genomes will facilitate a better understanding of miRNA evolution. An emerging model species of the Camelineae and the Brassicaceae is Capsella rubella, which closely related to Arabidopsis thaliana and Arabidopsis lyrata. Here we describe the miRNA complement of C. rubella. In addition to verifying miRNAs conserved between C. rubella and A. thaliana, we identify new high-confidence miRNA candidates specific to the C. rubella lineage. We examine conservation of miRNAs and their targets between the three Camelineae species, C. rubella, A. lyrata and A. thaliana. miRNAs of the 20/21nt class are most deeply conserved and have significantly lower divergence than those of the 22nt size class that are less evolutionarily conserved. Most targets of miRNAs are predicted in only a single species and not conserved across the Camelineae indicating the transitive nature of most miRNA-target pairings on an evolutionary time scale. We present additional results on the polymorphism of miRNAs and their targets in 80 resequenced accessions of A. thaliana.

WORK-05-02

RNA SECONDARY STRUCTURE AS A POTENT CIS-REGULATORY ELEMENT IN *ARABIDOPSIS*

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The functional structure of all biologically active molecules is dependent on intra- and inter-molecular interactions. This is especially evident for RNA molecules whose functionality, maturation, and regulation requires formation of correct secondary structure through encoded base-pairing interactions. We have recently used a high-throughput, sequencing-based, structure-mapping approach in conjunction with transcriptome-wide sequencing of polyA+-selected (RNA-seq), small (smRNA-seq), and ribosome-bound (ribo-seq) RNA populations to investigate the impact of RNA secondary structure on gene expression regulation in Arabidopsis. From this analysis, we found that RNA folding is significantly anti-correlated with overall transcript abundance, which is likely due to the increased propensity of highly structured mRNAs to be degraded and/or processed into smRNAs. In fact, our results suggest that processing of highly structured RNAs into smRNAs may be a significant posttranscriptional regulatory mechanism in Arabidopsis that regulates specific sets of mRNAs encoding proteins with related functions including RNA silencing and defense responses. Finally, we find that secondary structure affects translation, and is also significantly higher in regions of mRNAs encoding protein domains. In total, our findings suggest that this feature regulates plant gene expression at multiple levels in plants.

WORK-05-03

INHIBITION OF PLANT MICRORNA ACTIVITY USING MOLECULAR SPONGES WITH MULTIPLE MICRORNA BINDING SITES

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In both plants and animals, elucidation of microRNA (miRNA) function through a traditional loss-of-function approach has proven difficult due to extensive genetic redundancy among most miRNA families. To address this, technologies such as target MIMICs (MIMs) in plants or molecular SPONGEs (SPs) in animals have been used as transgenic approaches to generate loss-of-function phenotypes. They overcome redundancy by sequestering highly similar miRNA family members, thereby perturbing endogenous miRNA:target interactions. Here, we test whether SPs can inhibit miRNA activity in plants. Synthetic SPs transcripts with 15 miRNA binding sites separated by four nucleotide spacers and driven by the 35S promoter were designed to individually target the Arabidopsis miR159 and miR165/166 families. While SPs with wild-type miRNA binding sites have no apparent impact on miRNA regulation, SPs containing miRNA binding sites with two central mismatches (cmSPs) can generate very strong loss-of-function phenotypes. Comparison of the efficacy of the cmSPs to the corresponding MIMs finds that the efficacy of the different approaches varies; SP165/166 appears much stronger than MIM165/166, whereas MIM159 can generate stronger phenotypes than cmSP159. However, analysis suggests that MIM159 targets both miR159 and the closely related miR319 family, whereas cmSP159 appears specific for miR159. Therefore, in terms of efficacy and specificity, the use of cmSPs appears a practical technology for inhibiting miRNA function in plants. We argue that a key component of efficacy will be target site accessibility, and the use of multiple miRNA binding sites within a single transcript may be beneficial in this regard.

WORK-05-04

CALCIUM IS THE MOLECULAR SWITCH SHIFTING THE PHYTOSULFOKINE RECEPTOR 1 (PSKR1) FROM KINASE TO GUANYLATE CYCLASE ACTIVITY

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Many plant responses are mediated by interactions between intracellular calcium and the second messenger cGMP formed by guanylate cyclases (GCs). Previously we identified a novel class of receptor-GCs containing the GC catalytic center embedded within the kinase domain and showed that the recombinant cytoplasmic domain of phytosulfokine receptor AtPSKR1 has both guanylate cyclase and kinase activity in vitro (Kwezi et al. 2011 J Biol Chem 286: 22580-8). We now show that physiological increases in calcium levels enhance GC activity of AtPSKR1 whereas these calcium levels reversibly inhibit kinase activity. In addition PSKR1 kinase activity is reduced in the presence of the GC product cGMP. Recombinant AtPSKR1 can undergo in vitro autophosphorylation and we have confirmed it has 14 phosphorylation sites in its cytoplasmic domain including 8 serine, 3 threonine and 3 tyrosine residues. Three phospho-serine residues at the juxta-membrane position were mutated to either mimic phosphorylation on or off states. Kinase activity was enhanced in the on mutant and suppressed in the off mutant while GC activity was unaffected suggesting calcium acts as a molecular switch of PSKR1-mediated signalling that can be modulated by the phosphorylation state. The challenge now lies in understanding how molecular interactions between the GC and kinase domains are capitalized on in the plant.

WORK-05-05

ANCESTRAL FUNCTION OF CLE SIGNALING IN LAND PLANTS

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Small secretory peptides encoded by CLE(CLV3/ESR-related) genes play important roles in plant growth and development. The Arabidopsis CLE gene family consists of 32 members and some receptors for them have been identified. In the shoot apical meristem, CLV3 signaling is well known to regulate the size of the stem cells via a regulatory feedback loop involving WUSCHEL. The LRR receptor kinase, CLV1, and other LRR receptor genes are responsible for the perception of the CLV3 signal. In the vascular meristem, TDIF/CLE41 signaling is mediated by another LRR-RK, TDR, and regulates stem cell fates. However, the biological functions of the CLE genes are diverse and their signaling pathways seem to be complicated in Arabidopsis. In the moss Physcomitrella, we could find only 7 CLE genes and all of them are CLV3-like rather than TDIF-like. We further searched for CLE genes in the liverwort Marchantia and have found only 2 genes, which belong to TDIF-like and CLV3-like CLE genes, respectively. We also found CLV1-like and TDR-like genes in Marchantia EST databases, suggesting that both TDIF-like and CLV3-like CLE signaling was present in the last common ancestor of extant land plants. Overexpression of CLV3-like MpCLE2 affected growth and development of Marchantia thallus (gametophytic generation). Further molecular genetic analysis on MpCLE2 is ongoing and will be reported.

WORK-05-06

REGULATORY PEPTIDES THAT CONTROL ROOT DEVELOPMENT IN RESPONSE TO ENVIRONMENTAL CUES

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Plant root architecture requires coordinated regulation of endogenous developmental programs and environmental stimuli. It is known that phytohormones control many aspects of root development, however it has only recently been reported that small secreted peptides are implicated in aspects of root development including meristem maintenance, gravitropism and lateral root development. Here, we describe a regulatory peptide that affects several aspects of root development and is induced in the root tip by environmental cues, particularly nitrate starvation and high salt. Upon overexpression, or exogenous application of the peptide, there is a strong reduction in the overall size of the root system. Lateral root formation is perturbed at an early stage and primary root growth is dramatically slowed. A T-DNA insertion mutant shows the opposite phenotype, producing a larger root system, particularly under nitrate limitation and salt stress. Our work suggests a role for this peptide as a negative regulator of root development and provides a link between developmental programs and environmental stimuli.

WORK-06-01

SO MANY SMART WAYS TO DIE – PROGRAMMED CELL DEATH IN PLANTS

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Death of individual cells or cell populations is crucial for life and development of complex multicellular organisms. Cell death decisions are tightly regulated by complex molecular mechanisms generally referred to as programmed cell death (PCD) control. In animals, the molecular control of PCD has been intensively studied to better understand PCD-related diseases such as cancer, autoimmune defects, and neurodegenerative diseases. In plants, however, little is known on PCD mechanisms so far, though correct prevention or execution of PCD is critical for plant development and for the plant's interaction with its environment. In this workshop we gather international experts of Arabidopsis PCD research in different cellular and biological contexts: PCD processes elicited by biotic and abiotic interactions with the environment, as well as PCD instances that are part of the plant's developmental program. In my introductory presentation, I will give a short glimpse on recent progress in PCD research and PCD concepts in plants.

WORK-06-02

CHARACTERIZATION OF *ARABIDOPSIS* INHIBITOR OF APOPTOSIS (IAP)-LIKE PROTEIN LACKING A BACULOVIRUS IAP REPEAT (BIR) DOMAIN PLAYS ROLE IN CELL DEATH PATHWAY IN PLANT AND ANIMAL SYSTEMS

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The sequence homology search for inhibitor of apoptosis (IAP) proteins resulted in identification of Arabidopsis IAP-like protein (AtILP) which was characterized by a C-terminal RING finger domain. The IAP family proteins showed a baculovirus IAP repeat (BIR) domain required for antiapoptotic activity which was absent in AtILP. The expression of AtILP in HeLa cells conferred resistance against tumor necrosis factor (TNF)-/ActD-induced apoptosis through the inactivation of caspase activity. The N terminal region did not show homology with known BIR domain but still involved in inhibition of caspase 3 activity invitro and blocked (TNF)-/ACtD-induced apoptosis, whereas C-terminal RING finger domain failed to inactivate caspase-3. The antiapoptotic activity of the AtILP N-terminal domain observed in plants was reproduced in an animal system. The overexpression of AtILP in Arabidopsis results in suppression of cell death in plants when treated with apoptosis inducer like fungal toxin fumonisin B1. The antiapoptotic activity of AtILP was due to inhibition of caspase activation and DNA fragmentation. Overexpression of AtILP also attenuated effector protein-induced cell death and increased the growth of an avirulent bacterial pathogen. In summary, we characterized a novel plant IAP-like protein lacking BIR domain which prevents caspase activation in Arabidopsis and showed that a plant anti-apoptosis gene has conserved function in both plants and animal systems.

WORK-06-03

A LIFE-OR-DEATH DECISION: INTRACELLULAR SIGNALING IN THE PLANT UNFOLDED PROTEIN RESPONSE

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Protein folding is a fundamental process in the eukaryotic cell. When unfolded or misfolded proteins accumulate in the endoplasmic reticulum (ER), a conserved response from yeast to mammals and plants called the unfolded protein response (UPR) is elicited to ensure proper protein folding by either enhancing folding capacity or attenuating folding demands. It is associated not only with many diseases in human but also environmental stress tolerance in plants. The unmitigated ER stress also promotes programmed cell death (PCD), which kills the unwanted cells to protect other cells in an ER-stressed environment. Previously we have identified two membrane-associated transcription factors, bZIP28 and bZIP60, that are important for the UPR signaling and gene regulation. Upon ER stress, bZIP28 is relocated from ER to Golgi where it is proteolytically activated and subsequently enters the nucleus to form a transcriptional complex with nuclear factor Y (NF-Y) subunits for downstream gene regulation. Here we have shown that the ER lumen-facing domain contains ER retention signal and is critical for the ER-to-Golgi movement. bZIP60 is also a type II membrane protein, its activation in UPR requires IRE1-regulated unconventional splicing in the cytoplasm at mRNA level. Both bZIP28 and bZIP60 up-regulate the expression of another membrane-associated transcription factor PEP (for programmed cell death promoter) in UPR in Arabidopsis. Loss-of-function mutants of PEP are more tolerant to ER stress than the wild-type control. Following ER stress, PEP is relocated from the ER membrane to the nucleus. Conditionally overexpression of PEP induces PCD and activates several known PCD genes and components involved in chromatin remodeling and histone modification. Thus, ER stress activates a set of membrane-associated transcription factors to promote not only cell survival but also cell death. How the pro-survival and pro-death signals are integrated to decide the life-or-death cell fate remains future challenge.

WORK-06-04

STAX, A NOVEL NEGATIVE TRANSCRIPTIONAL REGULATOR OF ARABIDOPSIS LEAF SENESCENCE

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

WORK-06-05

VND7-BINDING SEQUENCES REVEALED BY FLUORESCENCE CORRELATION SPECTROSCOPY

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The Arabidopsis thaliana NAC domain transcription factor, VASCULAR-RELATED NAC-DOMAIN7 (VND7), acts as a key regulator of xylem vessel differentiation. Our previous study revealed a large number of putative direct target genes of VND7, which encode a broad range of proteins, such as transcription factors, IRREGULAR XYLEM proteins and proteolytic enzymes including XYLEM CYSTEINE PROTASE 1 (XCP1). Moreover, at least two distinct regions in XCP1 promoter responsible for VND7 binding, X1E1 and X1E2, were identified by a promoter-deletion analysis and an electrophoretic mobility shift assay (EMSA). However, cis-elements for VND7-binding are still not fully understood. Therefore, in this study, we attempted to identify cis-elements of VND7 using a new technique with Fluorescence Correlation Spectroscopy (FCS) which allows us to characterize the molecular-molecular interaction quantitatively on a large scale. As a result, FSC successfully detected the binding between a fluorescence (TAMRA)-labeled X1E1 (TAMRA-X1E1) and NAC-domain of VND7 fused with maltose binding protein (MBP) (MBP-VND7(NAC)). In addition, the excess amount of fluorescence-free X1E1 completely competed with the TAMRA-X1E1, suggesting the FCS is comparable to the EMSA for the analysis of binding between cis-elements and transcription factors. We finally succeeded in narrowing down the binding sequence from 53 bp to 18 bp with the deletion- and point mutation-versions of fluorescence-free competitors. We are now searching cis-elements in the other direct target genes of VND7 and of other related NAC transcription factors associated with xylem cell differentiation, which will allow us to better understand the molecular mechanisms of vascular development.

WORK-06-06

THE OUTER MITOCHONDRIAL MEMBRANE AAA ATPASE *BCS1* IS INVOLVED IN PATHOGEN RESISTANCE

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Plants are continuously exposed to adverse external factors and must respond appropriately to survive. One of the most widely stress responsive genes that encodes a mitochondrial protein is BCS1. BCS1 responds to a range of stresses including abiotic stresses, mitochondrial and chloroplast dysfunction, and also pathogen infection. The closest homolog of BCS1 in animals encodes an inner mitochondrial membrane chaperone involved in Complex III assembly and is associated with multiple heritable illnesses. In plants, BCS1 encodes an outer mitochondrial membrane protein belonging to the AAA-type ATPase protein family. Although transgenic plants with reduced BCS1 expression look phenotypically normal, BCS1 overexpression lines have a distinct phenotype. The plants are slightly smaller, show strong leaf curling and have increased starch content. Analysis of mitochondrial protein content demonstrated no obvious changes in mitochondrial respiratory complex protein abundance. In line with the stress inducible expression pattern BCS1 overexpression lines are more tolerant to drought stress. Furthermore, BCS1 overexpression plants are more tolerant to the biotrophic pathogen Pseudomonas syringae, whereas they are more susceptible to the necrotrophic fungus Botrytis cinerea, suggesting a possible role for BCS1 in regulating cell death. We have identified a number of interacting proteins using immuno-precipitation that provide further insight into the molecular function of BCS1.

WORK-07-01

INTRODUCTION

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WORK-07-02

HIGH-THROUGHPUT, IMAGE-BASED ANALYSIS OF TRAITS IN MODEL PLANTS

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Streamlining the discovery of plant traits highly relevant to pressing agricultural problems in Australia and worldwide, such as salinity, drought and disease tolerance is paramount to meet projected global food demands under extreme weather in coming decades. This is transitioning plant physiology research from manual studies of a few different genotypes, to high throughput, quantitative, temporal studies of large populations of plants with fully sequenced genomes. Today, high throughput phenotyping (HTP) is an emergent field which seeks to quantify the multi-dimensional basis of plant phenotypes on a large scale using non-invasive technologies. When HTP is combined with population-wide genotyping, the genetic architecture of correlated traits can be unravelled. Yet for these techniques to be widely used, it is necessary to automate the capture and processing of image data into quantitative values. In this presentation, we introduce two new high-throughput phenotyping platforms, CabScan™ and Trayscan™, associated with a complete image analysis pipeline for analyzing growth and function of model plants in 2D, 3D and 4D. The Cabscan™ phenomics platform is an in-cabinet imaging robot platform, using visible stereoscopy and infrared imaging, to monitor the growth and function of 320 plants at once. The system enables one to scrutinize plant growth with high spatial and high temporal resolution up to four times per hour, day and night, seven days a week. The growth chamber, in which the robot operates, is also equipped with a multi-wavelength LED-light enrichment system, thus providing a high level of spectral control. The Trayscan™ platform is an automated phenotyping platform equipped with a conveyor system enclosed in an acclimation room for allowing control of light and temperature. This system has a throughput of 2400 plants per day, is equipped with an automatic watering station for applying controlled drought stress, and provides image data from infrared, visible and pulse modulated chlorophyll fluorescence cameras.

WORK-07-03

HIGH THROUGHPUT PHENOTYPING OF MODEL PLANTS FOR BIOMASS ACCUMULATION AND PHOTOSYNTHETIC EFFICIENCY TRAITS

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TrayScan was developed from a partnership between CSIRO HRPPC and Photon System Instruments (Czech Republic) as a high throughput phenotyping platform designed specifically for monocot and dicot model plants and small seedlings. TrayScan can scan up to 15 trays of 20 plants per load and estimate within minutes, architectural parameters such as projected leaf area from 3 positions in space and also access physiologically relevant indicators such as canopy temperature and chlorophyll fluorescence. This is made possible by the use of several RGB cameras, thermal infrared (FIR) and a chlorophyll fluorescence system. The platform allows controlling light and temperature conditions to pre-acclimatise the plants, is fully programmable and automated for the data acquisition process. Plants and trays are bar-coded, data and metadata are stored on a data base for subsequent analysis and mining. We illustrate here the performance of the platform using Brachypodium distachyon, a model plants for grasses and cereals. The genome of Brachypodium distachyon has been fully sequenced and a T-DNA mutant library has been generated by our partners in the US and sent to HRPPC for phenotyping. We are investigating robust performance traits such as biomass accumulation and growth rates. We are also interested in the physiology of the plants through studies of traits involved in photosynthesis (stomatal conductance and photochemical efficiency) that can be used as proxies for biomass screening. This population is homozygous and will allow linking the phenotypes observed to a genomic region. Brachypodium distachyon is sharing a high degree of homology and synteny with food crops and energy crops such as wheat and miscanthus.

WORK-07-04

POSITIONAL CLONING OF A PROTEIN KINASE INVOLVED IN NA+ EXCLUSION IN *ARABIDOPSIS*, LEADING TO IMPROVED SALT TOLERANCE IN BARLEY IN THE FIELD

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Salinity is a major abiotic stress which affects crop plants in Australia, resulting in substantial loss of yield and millions of dollars of lost revenue. High levels of Na+ in shoot tissue have adverse osmotic effects and reduce the amount of K^+ available for essential biological processes. Crucially, yield in cereals is commonly inversely proportional to the extent of shoot Na^+ accumulation. Through the use of an Arabidopsis thaliana mapping population we have identified a highly significant QTL linked to Na+ exclusion. Fine mapping of this QTL identified a protein kinase (AtCIPK16) that was significantly up-regulated under salt stress. Constitutive over-expression of the gene in Arabidopsis, rice and barley leads to plants with significant reduction in shoot Na+ and greater salinity tolerance. Transgenic barley over-expressing AtCIPK16 were grown in a saline field trial site for the first time in 2012. Under high saline conditions the transgenic barley had reduced shoot Na^+, increased biomass and maintained higher grain yield than non-transgenic barley.

WORK-07-05

GENETIC DISSECTION OF PLANT DEVELOPMENT USING GWAS AND QTL ANALYSES IN *ARABIDOPSIS THALIANA*

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Natural variation is an important source for the identification of quantitative trait loci (QTLs) in experimental populations. Genome-wide association studies (GWAS), in which natural populations are used, profit from much higher resolution and were expected to fill the gap between the detection of QTLs and the identification of causal genes. To date, however, only few GWAS have led to the identification of novel causal genes for a multitude of traits studied in Arabidopsis thaliana. In our study, we have analysed a diverse collection of 350 Arabidopsis accessions for many growth and development related phenotypes. The different ecotypes showed a wide variety in growth rate, leaf shape and shoot architecture. Additionally, we analysed a number of enzymes, structural components and metabolites in carbon metabolism and found substantial differences between the accessions. Most enzymes and metabolites were found to correlate negatively with biomass, suggesting that fast growing plants have a higher metabolic flux rate. For the majority of traits, strong candidate genes could be assigned using standard association mapping approaches. Different traits were sometimes found to associate with the same genomic region, suggesting a major role for the underlying gene in the regulation of the biological pathway. For many of the phenotypes, the results were compared to low resolution mapping in a previously analysed bi-parental recombinant inbred line (RIL) population. Although some overlap was found, the majority of significant associations did not coincide with QTLs detected in the RIL population, most likely due to the higher allelic diversity in the association panel. A number of such novel significantly associated loci are currently being functionally characterized in molecular and physiological follow-up studies.

WORK-08-01

HOW TO BE A GREAT TEACHER: TIPS AND RESOURCES FOR PLANT SCIENTISTS

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What does it take to be an effective university teacher? What resources are available to help plant scientists teach effectively? What does the science of teaching and learning tell us about best practices to enhance student learning? Can you be a good teacher and a successful researcher? This workshop is designed to introduce basic higher-education teaching skills to early career plant biologists. The workshop includes short presentations, but primarily is interactive and allows participants the opportunity to engage in, plan and practice diverse forms of teaching and learning. Topics include defining learning objectives, inquiry-based learning, scientific teaching, and integrating research into teaching. Participants will be given PDF packets of materials and resources discussed in the workshop.

WORK-08-02

ARABIDOPSIS DETECTIVES: INNOVATIVE APPROACH TO RESEARCH-LED DRIVEN TEACHING

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The next generation of plant science graduates will need creativity backed by high quality knowledge and investigative skills if they are to tackle the challenges of food production and biodiversity management in the face of climate change. Plants: Genes to Environment (BIOL2121) is the key course introducing plant science to undergraduates at the Australian National University. This research-led course features an interactive approach by research-active staff, and innovations such as peer-assisted learning in lectures, the inquiry-based identification of Arabidopsis thaliana mutants in practical classes, the support of previous year students as Peer Mentors, and engaging research-based approaches to assessment. Arabidopsis is a powerful species with which to teach the basic principles of plant physiology and genetics because of the comprehensive understanding of its physiology and genetics, and an extensive collection of mutants and protocols. In the Plant Detectives project, teams of students put into practice their newly acquired theoretical knowledge as they apply cutting-edge laboratory techniques to identify Arabidopsis mutants. In describing the award-winning course's innovative design and multiple positive outcomes, we shall show how we believe the future of plant science research lies in engaging today's students as researchers and how one small plant is helping us do this.

WORKSHOP 9 – PLANT NUTRITION IN THE FACE OF IMPENDING GLOBAL RESOURCE LIMITATION OPPORTUNITIES FOR MODEL PLANT RESEARCH

Sponsored by University of Western Australia

WORK-09-01

TOO MANY VARIABLES - HOW TO CHOOSE PARAMETERS FOR NUTRIENT SIGNALLING EXPERIMENTS

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Justus Liebig's principle of the limiting factor is a well-known concept for optimizing plant nutrition. It is also widely used when assessing the importance of individual nutrients and their effect on transcriptional networks. There is evidence, however, that some traits (e.g. root growth) are also affected by the excess of other nutrients (e.g. iron) that accumulate upon the omission of the target nutrient (e.g. phosphate). These effects are highly dependent on the context – i.e. the overall nutrient composition of the growth medium – and therefore are hard to reproduce across experimental set-ups. Since plant scientists are increasingly interested in the characterization of the cross-talk between (macro-) nutrient signalling pathways, it becomes more important to standardize experimental approaches. Software tools that compute the chemical speciation for a given nutrient solution can help to estimate ionic interactions between nutrients and avoid selective precipitation. Other factors known to impact on experimental outcomes include the composition of gelling agents, types of sealing film, day length, light intensity, sucrose addition and exclusion of light from the root zone for plate experiments as well as the frequency of nutrient solution exchange, plant-to-volume ratios, silicate addition, aeration and light conditions for hydroponic set-ups. Aside from these general considerations, the use of common markers for different nutrient stress pathways across experiments could help to interpret findings and put them into a more general nutritional context. A first list of these 'nutrient balance reference genes' will be presented for discussion and expansion by the audience.

WORK-09-02

REGULATION OF HIGH AFFINITY PHOSPHATE TRANSPORTERS IN *ARABIDOPSIS*

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Phosphate (Pi) is a crucial and often limiting nutrient for plant growth. It is also a very insoluble ion heterogeneously distributed in soil. The uptake of this element relies on the presence of multiple high affinity transporters (PHT1 family) located in plasma membranes (1). Multiple and complex steps of regulation of these proteins were identified illustrating the capacity for plants to tightly control the level of these transporters into the cells (2,3,4,5). Radioisotope live micro-imaging system (6) was used to image spatial distribution of Pi absorption along the root. Combined with various genetic manipulation approaches to manipulate this broad family of transporters, it reveals unexpected location of absorption and provides opportunity to dissect the function of redundancy of this multigenic family. 1 Nussaume et al. (2011). Front Plant Sci. 2 Misson et al. (2004). Plant Mol Biol. 3 Bayle et al., (2011). Plant Cell 4 Thibaud et al., (2010). Plant J. 5 Misson et al. (2005). PNAS USA. 6 Kanno et al. (2012) Philos Trans R Soc Lond B Biol Sci.

WORK-09-03

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL MECHANISMS FOR HOMEOSTATIC CONTROL OF SULFATE TRANSPORT AND ASSIMILATION IN PLANTS

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Sulfur is essential for plant growth and fitness. The metabolic pathways for sulfate assimilation are regulated by supply of sulfate and demands for production of reduced sulfur compounds in plants. They are suggested to be regulated homeostatically under highly controlled mechanisms in which the functions of a central transcriptional regulator, SLIM1, and its downstream target genes are involved. In Arabidopsis, SLIM1 is primarily responsible to induce mRNA accumulation of sulfate transporters facilitating the import of sulfate across the plasma membranes in root epidermal and cortical cells and releasing sulfate from the vacuoles under sulfur-limited conditions. SLIM1 can also induce accumulation of microRNA-395 (miR395) which specifically targets on mRNAs encoding plastid-localizing ATP sulfurylase (ATPS1, ATPS3 and ATPS4) and low-affinity sulfate transporter, SULTR2;1, and abolishes their functions in sulfur metabolism and internal sulfate translocation in response to sulfur deficiency. It is suggested that miR395 essentially limits the flux of sulfate assimilation in plastids and helps plants to allocate sulfate to source leaves. In this workshop, we will present the results from our recent study on metabolic flux regulation of sulfate assimilatory pathways and its relationships with transcriptome-wide responses of sulfur-regulated gene networks in Arabidopsis.

WORK-09-04

NITRATE SENSING AND SIGNALING IN *ARABIDOPSIS THALIANA*

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Nitrogen (N) is one of the key mineral nutrients for plants and its availability has a major impact on their growth and development. The main N sources for terrestrial plants in soils is nitrate (NO_3^-) and most often it is a growth limiting factor. Plants are able to sense NO_3^- in their environment, allowing them to quickly respond to the dramatic fluctuations of its availability. Significant advances have been made during the recent period concerning the molecular mechanisms of NO_3^- sensing and signalling in higher plants. The striking action of NO3- as a signal molecule on genome expression has been unravelled. Note worthily, NO_3^- sensing systems have been identified. These unexpectedly correspond to membrane transporters also ensuring the uptake of NO_3^- into root cells, thus generalizing the nutrient 'transceptor' (transporter/ receptor) concept defined in yeast. Furthermore, components of the downstream transduction cascades, such as transcription factors or kinases, have also been isolated. Recently, a major breakthrough arising from this improved knowledge is a better understanding of the integration of NO_3^- and hormone signalling pathways, that explains the extraordinary developmental plasticity of plants in response to NO_3^-.

WORK-09-05

ROLES OF UBIQUITINATION IN THE CONTROL OF PHOSPHATE STARVATION RESPONSES IN ARABIDOPSIS

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Given the importance of the macronutrient phosphorous as part of key molecules and as a metabolic regulator, maintaining phosphate (Pi) homeostasis is critical for plants survival. Plants have developed multiple adaptive regulatory mechanisms that control the continuous perception and integration of information on local and whole plant Pi status, and, therefore, the coordination of the adaptive responses to Pi deprivation (-Pi) (optimization of Pi uptake, mobilization and distribution, and protection against -Pi stress adverse effects). These mechanisms include both the regulation of gene expression, which is greatly orchestrated by PHR1/PHL1 transcriptional factors, and the post-transcriptional control of gene product stability and function. The latter is mainly driven by targeted protein degradation in which ubiquitination plays a fundamental role. Recent studies have highlighted the relevance of ubiquitination and ubiquitin (Ub)-deconjugation pathways in the control of –Pi adaptive responses including root and root hair development, maintenance of Pi homeostasis, transcriptional control of Pi starvation responsive (PSR) genes and accumulation levels of potential Pi signaling proteins, among others. These mechanisms can trigger substrate degradation by two different proteolytic machineries: the 26S proteasome and the endocytic/vacuolar protein sorting pathway. A variety of Ub/26S proteasome system (UPS) components are likely to be involved in the control of plant adaptation to low Pi-stress, specially the E3 Ub ligases (E3s) that specifically recognize target proteins and facilitates their covalent modification with Ub. Future studies will unquestionably shed light on the contribution of the UPS in the regulation of Pi signaling and -Pi responses. In agreement, we will discuss on our latest results in the analysis of variation in the nuclear proteome of Arabidopsis that depends on -Pi and the UPS, as well as, on the functional characterization of Pi-responsive E3s.

WORK-09-06

UNRAVELING SIGNALING PATHWAYS INVOLVED IN NUTRIENT ACQUISITION VIA METABOLOMICS AND SYSTEMS BIOLOGY DRIVEN APPROACHES

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Forward and reverse genetics have accelerated the number of functional gene annotations made. However, signal transduction mechanisms, subjected to post-translational modifications, are much more tedious to decipher using these methods. One alternative approach is to profile the metabolite complement of the cell. As the chemical modulators in metabolism, it provides a more comprehensive overview of post-transcriptional and –translational events associated with the early phosphate starvation response. Here an unbiased, untargeted metabolome approach using a range of extraction and mass spectrometry methods was employed to monitor the chemical response upon phosphate starvation and re-supply conditions in Arabidopsis and rice. Metabolite analyses, combined with computational and empirical approaches have uncovered i) specific underlying patterns, ii) altered progression and iii) unique dynamics of the phosphate-responsive metabolome. Chemical annotation and functional characterization of these metabolites have been performed. As proof of concept, phosphate friendly 1, a metabolite that accumulate upon phosphate starvation, alter the root system architectural (RSA) response under phosphate sufficient conditions to elicit the same type of response as those roots grown upon phosphate starvation. Furthermore, phosphate friendly 1also alter RSA responses during N limitation. The results will be discussed in the context of the elucidation of the biosynthetic pathway of phosphate friendly 1 in plants, as well as the transcriptional and post-transcriptional regulators identified thusfar. This work provides novel insight into root growth and development underlying nutrient acquisition strategies.

WORK-10-01

INTRODUCTION: PROTEOMIC RESOURCES AND THE ARABIDOPSIS PROTEOMICS SUBCOMMITTEE

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The proteomics subcommittee of the Multinational Arabidopsis Steering Committee was established to facilitate the coordination of international research in Arabidopsis thaliana in the area of proteomics. The subcommittee (MASCP) is comprised of over twenty international researchers with extensive experience in the field of plant proteomics. Members have been highly active in the development and integration of tools and databases to enable proteomics-based research in Arabidopsis. A brief overview of the goals of the subcommittee will presented as well as Arabidopsis proteomic resources and tools that are currently available to the community.

WORK-10-02

INTEGRATING GENETICS AND PHOSPHOPROTEOMICS REVEALS A PROTEIN PHOSPHORYLATION NETWORK IN THE ABSCISIC ACID SIGNALING PATHWAY IN ARABIDOPSIS

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Abscisic acid (ABA) is a phytohormone that regulates diverse plant processes, including seed germination and the response to dehydration. In the major ABA signaling pathway, three members of SNF1-related protein kinase 2 (SnRK2) family transmit ABA-induced signals through phosphorylation of downstream substrates. To identify such substrates, we screened thousands of phosphoproteins in Arabidopsis by mass spectrometry-based phosphoproteomics. We identified proteins that were phosphorylated in Arabidopsis wild-type plants, but not in mutants lacking SnRK2s (srk2dei), treated with ABA or subjected to dehydration stress. Comparative analysis revealed that 35 peptides were differentially phosphorylated in wild-type but not in srk2dei plants. Biochemical and genetic studies of candidate SnRK2-regulated phosphoproteins showed that SnRK2 promoted the ABA-induced activation of MAPK(s), AtMPK1/2; that SnRK2 mediated phosphorylation of Ser^{45} in a bZIP transcription factor, AREB1, and stimulated ABA-responsive gene expression; and that a previously unknown protein, SnRK2-substrate 1 (SNS1), was phosphorylated in vivo by ABA-activated SnRK2s. Reverse genetic analysis revealed that SNS1 acts as a negative regulator of ABA responses. Thus, by integrating genetics with phosphoproteomics, we identified multiple components of the ABA-responsive protein phosphorylation network. (Umezawa et al. Sci. Signal. 6: rs8, 2013).

WORK-10-03

REDOX-REGULATION OF THE SUMO E2 IN PLANT IMMUNITY

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Post-translational modification (PTM) of proteins vastly increases the complexity and functional diversity of the proteome to precisely regulate crucial cellular systems. Many of these modifications involve small, redox-active molecules and occur after the rapid synthesis of reactive oxygen intermediates (ROIs) and nitric oxide (NO) associated with immune activation. Another key PTM is SUMOylation, an essential mechanism involving the conjugation of the small ubiquitin-like modifier (SUMO) to target proteins, regulating a myriad of cellular processes. The targets and mechanisms of SUMOylation are now emerging, although how this PTM is regulated remains largely unknown. Thus, we investigated if components of the Arabidopsis SUMOylation machinery are regulated by redox-based modifications, and whether this may be involved in plant immunity. We have discovered that a previously uncharacterised cysteine in SUMO-conjugating enzyme 1 (SCE1) might have an important role in the redox-regulation of this key enzyme during plant immune function.

WORK-10-04

PROTEOME AND METABOLOME PROFILING OF CYTOKININ ACTION IN *ARABIDOPSIS* IDENTIFYING BOTH DISTINCT AND SIMILAR RESPONSES TO CYTOKININ DOWN- AND UP-REGULATION

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Ectopic over-expression of genes involved in cytokinin (CK) biosynthesis (ipt) and degradation (CKX) has dramatically contributed to defining roles of CKs in plant growth and development regulation. Molecular mechanisms underlying their regulatory roles have been intensively researched. However, proteomic and metabolomic responses to CK deficiency are unknown. Therefore, we have compared global responses at these levels to reductions and increases in the bulk CK pool in Arabidopsis seedlings, mediated by inducible barley cytokinin oxidase/dehydrogenase and Agrobacterial isopentenyltransferase constructs, respectively. Proteomic analysis identified >1100 proteins, 155 of which responded to HvCKX2 and/or ipt activation and are mostly involved in growth, development and/or hormone and light signaling. The metabolomic analysis covered 79 metabolites, 33 of which responded to HvCKX2 and/or ipt activation and included mostly amino acids, carbohydrates and organic acids. Comparison of the datasets revealed unexpectedly extensive overlaps, of 31% and 12% of differentially regulated proteins and metabolites, respectively. Processes represented in the overlaps are mainly linked to growth and development, photosynthesis and carbohydrate metabolism, and may explain some surprising similarities found in previous experiments between plants with increased and decreased CK levels. Further, integration of our data revealed novel components of molecular circuits involved in cytokinin action; unrecognized links to redox regulatory network and signaling of stress hormones; and markers of cytokinin content. This work was supported by grant P305/12/2144 (GACR) and ESF project 'Postdocs in Biological Sciences' (CZ.1.05./1.100/02.0068).

WORK-10-05

PROTEOMICS OF MODEL PLANT SYSTEMS TO IDENTIFY MECHANISMS ESSENTIAL FOR PLANT SALT TOLERANCE

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Salinity is a major limiting factor for plant growth and agricultural productivity. In the lab we have been using the model glycolytic plant Arabidopsis thaliana as well as salt-tolerant model plants, including Thellungiella salsuginea (Thellungiella halophila) and the halophyte Mesembryanthemum crystallinum, to study adaptive mechanisms important for plant salt tolerance, with particular emphasis on membrane proteins. Studies show that both Arabidopsis and Thellungiella accumulate Na^+ in older leaves; in contrast, M. crystallinum selectively sequesters Na^+ in young, actively growing tissues and in specialized epidermal bladder cells. While strategies to deal with the increasing cellular salt concentration differ, all species apparently rely on similar membrane transport mechanisms to distribute cellular Na^+; including tonoplast H^+-ATPase (VHA), Na^+/H^+ exchangers (NHX's), and Na^+/K^+ transporters (HKT's) to name a few; although expression and activity levels of the transporters appears to differ. This suggests that understanding possible species specific regulation, and identifying proteins that interact with these transporters, may increase our insight into the complex mechanisms used to enhance salt tolerance. To accomplish this we have been exploiting Free Flow Electrophoresis to fractionate cellular membranes from the above mentioned species in parallel with quantitative proteomics methodologies, using both in-gel label- and label-free LC-MS/MS approaches. Results from these studies will be presented and discussed. Authors would like to acknowledge UNAM-DGAPA-PAPIIT (IN203913 to BJB, IN207311 to RV-E) and CONACyT (79191) for funding proteomic studies in the lab.

POSTERS

POS-TUE-001

WILD POPULATIONS OF *ARABIDOPSIS THALIANA* FROM SOUTH AMERICA: A STUDY OF PHYSIOLOGY AND GENETIC DIVERSITY

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To compare the physiological and genetic characteristics of Arabidopsis thaliana plants growing in South America with those of more extensively-studied geographic regions, we collected *A. thaliana* plants from four ecologically diverse sites in Patagonia, Argentina. In the wild, these plants had a rapid spring-summer reproductive cycle and exhibited variation in growth morphology. In the lab, the Patagonia plants were less sensitive to red light signaling, had a better temperature compensation mechanism for the circadian rhythm of leaf movement, and exhibited a constitutive shade avoidance response during growth under different light conditions compared to Col-0. They were late-flowering in both long and short photoperiod conditions, but flowered much earlier after vernalization. The vernalization requirement was correlated with *FLC* expression and could be overcome by growth in constant white light at 10°C. We examined the genetic diversity of Patagonia accessions at a coarse scale relative to 5500 worldwide accessions using a clustering homology analysis of 149 SNPs and at a fine scale using whole-genome sequencing of four Patagonia individuals compared to 80 Eurasian accessions. Genome-wide analysis at the coarse scale showed that the Patagonia individuals belonged to the same haplogroup and were most similar to haplogroups from Italy. Comparable results were seen at the fine scale, with site-specific SNPs being rare. We conclude that the Patagonia accessions are a genetically uniform group that likely resulted from anthropogenic introduction from Europe. Although the Patagonia accessions exhibited similar physiological responses to different conditions in the lab, high phenotypic plasticity of Patagonia plants in the wild make this collection useful for characterizing genotype by environment interactions.

POS-WED-002

USING BAYESIAN METHODS TO REVEAL THE EVOLUTIONARY PATTERNS OF MALE FERTILITY RESTORER *(RF)* GENES

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The goal of this project is to identify 'restorer to fertility' (*Rf*) genes in multiple crop species. *Rf* genes are commercially important in plant breeding for cost effective hybrid seed production. *Rf* proteins suppress the action of mitochondrial genes that induce cytoplasmic male sterility (CMS), generally by binding to and preventing the expression of transcripts from CMS genes. *Rf* genes are difficult to map genetically due to the presence of clusters of related genes in close proximity, so we evaluated a novel bioinformatic approach for identifying them based on phylogenetic analyses of genome sequences. The in silico process for identifying *Rf* genes will be presented as well as an updated phylogeny derived from *Rf* genetic data from multiple species.

POS-TUE-003

LOOKING AT PATTERNING FROM AN EVOLUTIONARY PERSPECTIVE

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Evolutionary developmental biology can be employed to dissect the mechanistic understanding of phenotypic changes. Presently, in developmental biology, evolutionary comparisons comprise of a few well-characterized model systems separated by large evolutionary distances leading to a descriptive view rather than having a functional depth. Therefore, studying homologous processes in related species of the same taxon is required to obtain functional evolutionary comparisons of developmental processes. We apply this approach to explore the evolution of trichomes and root hair development by comparing these processes between *Arabis alpina* and *Arabidopsis thaliana*. *A.alpina* is a fitting eco-devo model to *A.thaliana* as it's a fairly distant member in the Brassicaceae family (~26 million years apart), diploid and self-compatible lines are available. In *A.thaliana*, both processes involve the same set of genes or closely related paralogs that control additional factors specific for the respective differentiation process. Several patterning genes exhibit molecular and mechanistic redundancy such that the same developmental outcome is driven by at least two regulatory circuits involving different gene sets. We apply forward genetics to uncover the mechanistic behind the evolution of patterning. Here we display the numerous patterning mutants revealed from the systemic screening of EMS mutagenized 30,000 M2 *A.alpina*. Alongside, we identify patterning genes in *A.alpina* by sequence analysis of the newly annotated genome, laying the foundations for a comprehensive study of evolution of a whole gene regulatory network.

POS-WED-004

THE GENOTYPE DATA ARE USED FOR PRESERVATION AND QUALITY CONTROL OF ARABIDOPSIS WILD TYPE STRAINS AND RELATED SPECIES AT RIKEN BRC

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RIKEN BioResource Center (BRC) takes over the project of the Sendai Arabidopsis Seed Stock Center (SASSC). The Arabidopsis wild-type strains and closed species are now preserved and distributed from BRC under the National Bio-Resource Project (NBRP) supported by the Japanese government (http://sassc.epd.brc.riken.jp/sassc/). We believe these Arabidopsis wild-type strains and closed species are indispensable materials for the research on evolution and adaptation to the environment. For the quality control of Arabidopsis wild-type strains and closed species seeds, we have tried to collect and apply their genotype data. For Arabidopsis closed species, we are collecting the DNA sequence of the chloroplast genome regions, rbcL and matK. These DNA sequences provide us the information on a classification of Arabidopsis relatives. For Arabidopsis wild-type strains, we use 16 SSLP markers. The data was obtained from each generation of seed stocks and used for the evaluation of their purity. Recently, we began to compare the genotype of the stocks among the centers using the SNPs markers. The data will be provided to the community through our database. We believe that the data will be useful for research communities.

POS-TUE-005

NATURAL VARIATION OF A GENE NETWORK REGULATING TRICHOME PATTERNING

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In *Arabidopsis thaliana*, the regulation of five different traits, namely trichome patterning, root hair patterning, seed coat mucilage formation, anthocyanin and proanthocyanin biosynthesis, is intertwined by an evolutionary conserved gene network coding for WD40, bHLH, MYB proteins.. These traits are likely to be naturally regulated as it has been shown that there are involved in protection against pathogens, UV, or germination (Calo *et al*, 2006; Yan *et al*, 2012). We are using a whole genome association mapping approach in *Arabidopsis thaliana* to analyse the diversity within the underlying network and to identify new genes contributing to these traits. We developed new screening techniques to analyze trichome patterning and proanthocyanidin content which allow rapid and precise phenotyping of Arabidopsis accessions. Recently, we released "TrichEratops", a software to analyze light microscopy pictures, allowing 3D reconstruction of the leaf surface and extracting known and new trichome patterning parameters. Even with low number of ecotypes (71) the first results show interesting candidates indicating that precise and fine phenotyping allow the use of genome wide association with a small batch of accessions.

POS-WED-006

COMPARATIVE ANALYSIS OF MIRNAS BETWEEN THE *BRASSICA RAPA* AND *ARABIDOPSIS THALIANA* GENOMES

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MicroRNAs (miRNAs) are one of the functional non-coding small RNAs involved in the epigenetic control of the plant genome. Although plant species contain both evolutionary conserved miRNAs and species-specific miRNAs within their genomes, computational methods often identify only evolutionary conserved miRNAs. We sought to provide a more comprehensive prediction of *B. rapa* miRNAs based on high throughput small RNA deep sequencing. We sequenced the small RNAs from the five tissues including seedlings, roots, petioles, leaves, and flowers. By analyzing 2.75 million unique reads that mapped to the *B. rapa* genome, we identified 216 novel and 196 conserved miRNAs that were predicted to target approximately 20% of protein coding genes in the genome. Comparative analysis of miRNAs between the *B. rapa* and *Arabidopsis thaliana* genomes demonstrated that redundant copies of conserved miRNAs in the *B. rapa* genome may have been deleted after whole genome triplication (WGT) whereas novel miRNA members seemed to arise spontaneously from both genomes suggesting species-specific expansion of miRNAs. We have made these data publicly available as a miRNA database of *B. rapa*, BraMRs. The database allows the user to retrieve miRNA sequences from the five tissues investigated as well as the expression profiles and description of their target genes. BraMRs will represent a valuable public resource with which to study the epigenetic control of *B. rapa* nd other closely related *Brassica* species.

POS-TUE-007

NATURAL VARIATION IN THERMAL RESPONSES OF ARABIDOPSIS

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Light and temperature are two key environmental factors that affect plant growth and development and there is extensive natural variation in the response of Arabidopsis strains to both these parameters (Alonso-Blanco et al., 2009). The effect of temperature has been well studied and characterized for low temperatures (0-8°C) and natural variation in vernalization (promotion of flowering in response to cold temperatures) is very well know. In contrast to light response, the physiological and developmental effects of small changes in the ambient temperature and hardly much is known about natural variation in ambient temperature responses. We have carried out QTL mapping analysis for key developmental traits in early and adult plant stages analyzing on one side the root and hypocotyls length and on the other side the flowering and related traits respectively. The systematic analysis of permanent populations like Recombinant Inbred Lines (RILs) has permitted us to identify genomic regions (QTLs) that could help to uncover the genetic architecture for the thermal response in Arabidopsis. We are validating and further characterizing some of these QTLs helping to unravel the genetic and molecular bases underlying different developmental traits affected by small ambient temperatures changes.

POS-WED-008

FUNCTIONAL ANALYSIS OF A CRYPTIC INTRON IN *ARABIDOPSIS THALIANA*

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Majority of primary transcripts arising out of a eukaryotic gene undergo splicing to generate the mRNA with the removal of introns. Often, there is variability in splicing due to the retention of introns, removal of exons, or the use of alternative splice sites. This process is referred to as alternative splicing. Higher temperatures modulate alternative splicing in *Arabidopsis thaliana*. We have previously shown that the *FLOWERING LOCUS (FLM)* gene plays a role in conferring natural variation in thermal response for flowering and this response is associated with changes in its splicing patterns (Balasubramanian et al, PLoS Genetics, 2006). Here, we report the discovery of a cryptic intron in the 3' UTR of the *FLM* gene that is spliced specifically only at higher temperatures. We have discovered a naturally occurring deletion precisely mapping this cryptic intron. The over expression of splice form lacking this cryptic intron failed to complement *flm* mutant, consistent with the previously described role of *FLM* in thermal response. One of the mechanisms through that precise intron deletions could occur involves reverse transcription mediated recombination. Computational analysis of intron deletions across multiple genomes did not suggest a 5'-3' bias indicating that other mechanisms may play a role. Recent progress on the functional and computational analysis will be presented.

POS-TUE-009

PHENOTYPIC VARIATION IN A LOCAL ISLAND POPULATION OF *ARABIDOPSIS THALIANA*

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The Bur-0 accession of Arabidopsis thaliana contains a natural intronic $(GAA)_n$ expansion in the ISOPROPYL MALATE ISOMERASE LARGE SUBUNT1 (ILL1) gene. The effects of this expansion result in cryptic growth defects, known as irregularly impaired leaf (iil) phenotype, only evident at higher temperatures. The Bur-0 accession originates from Burren, Ireland, a region renowned for its unusual assemblage of rare plant species owing to its unique glacio-karst limestone ecology. In order to determine whether this repeat expansion is maintained in the wild, a population genetic study was undertaken. Over 500 seed samples were collected from 200 separate locations across Ireland over a 2 year period. Analysis of wild accessions under different conditions revealed considerable variability in repeat lengths with several populations containing dramatically expanded $(GAA)_n$ tracts. This enables the analysis of triplet repeat mutational dynamics in the wild. A correlation was found between the severity of iil phenotype and the triplet repeat expansion. Demographic factors including geographic location, climate and landscape data were compared and the lines are currently being genotyped by sequencing. This study will improve our understanding on the genetic mechanisms underlying adaptive evolution in A. thaliana in Ireland as well as provide information on environmental conditions influencing $(GAA)_n$ repeat tract lengths.

POS-WED-010

TRIPLET REPEATS AND PHENOTYPIC VARIATION IN *ARABIDOPSIS THALIANA*

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Polymorphisms in microsatellites have been linked to phenotypic diversity in several prokaryotic and eukaryotic organisms. Expansions in triplet repeats underlie several neurodegenerative disorders in humans. We are using *Arabidopsis* as a model to investigate phenotypic variation conferred by repeats and its natural genetic modification. The *Arabidopsis* Bur-0 strain harbours a triplet repeat expansion in the *IIL1* gene, which confers a cryptic growth arrest phenotype (Sureshkumar *et al*, Science, 2009). In crosses with Col-0 this phenotype does not segregate as a monogenic trait suggesting the presence of modifiers. Using the Bur-0/Col-0 RILs, we have uncovered the genetic architecture of this modifier. While this repeat variability is in the introns, we have explored the variation in the exonic repeats by screening for genes that contain the repeats, which also are predicted to be polymorphic (Zeller *et al*, Genome Research, 2008). We have identified some interesting genes and we are currently investigating the phenotypic consequences of repeat variability in these genes. Recent progress on this analysis will be presented. Reference 1) Sureshkumar, S., *et al*, (2009) A genetic defect caused by a triplet repeat expansion in *Arabidopsis thaliana*. Science, 323:1060-1063 2) Zeller *et al*, (2008) Detecting polymorphic regions in *Arabidopsis thaliana* with resequencing microarrays. Genome Research, 18:918-929. .

POS-TUE-011

A CRYPTIC PHENOTYPIC VARIATION UNMASKED UNDER HIGHER TEMPERATURE IN *ARABIDOPSIS THALIANA*

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Cryptic genetic variation (CGV) is defined as genetic variation that does not contribute the normal range of phenotype, but can modify a phenotype after environmental challenge or the introduction of novel alleles or generation of new genetic combination after recombination. Although CGV is pervasive and might be an essential source of physiological and evolutionary potential, it is under-appreciated. Here we describe the genetic basis for a cryptic phenotype revealed under higher temperature in a wild strain of Arabidopsis. The Arabidopsis accession Sij-4, when grown at higher temperatures displays a very strong growth arrest phenotype. Fine mapping with 4000 mutant plants, we mapped the underlying loci to a 6-Kb region on chromosome 2, which includes only a single gene encoding the homologue of the yeast tRNA-Histidine Guanylyl transferase (THG1). In addition, T-DNA mutants in the Col-0 reference strains also displayed a strong temperature dependent phenotype resembling the Sij-4 growth arrest phenotype strongly arguing for THG1 to be the underlying gene. THG1 catalyzes a reaction to add guanosine to the 5' terminus of tRNA-Histidine. This G-1 addition step is a phosphodiester bond similar to the reactions catalyzed by DNA/RNA polymerases, but in the reverse 3'-5' direction. Recently, crystal structure of the human homologue of THG1 has been solved, which demonstrated a strong structural homology with polymerases suggesting THG1 could be a unique reverse polymerase. Moreover, compromising the activity of this protein in human cell lines led to cell cycle defects suggesting that this enzyme may play a role in cell cycle regulation. Now, we are testing the hypothesis of whether Arabidopsis Thg1 homolog plays a vital role in cell cycle dependent growth regulation at higher temperature.

POS-WED-012

ANALYSIS OF THE EPIGENOME IN ARABIDOPSIS HYBRIDS DURING EMBRYO DEVELOPMENT

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Hybrid progeny of two related species or two selected parental lines of the same species can display superior performance over the parents. This is referred to as Heterosis or Hybrid vigour. This phenomenon relies on the interaction between the two parental genomes and epigenomes, that result in the unique characteristics of the hybrid. Interaction occurs for the first time in the zygote, so it is plausible that the genetic and epigenetic instructions that lead to Heterosis later during development are established at this early stage. We are studying the early embryos generated from crosses between Arabidopsis ecotypes C24 and Landsberg erecta whose hybrids show heterosis for vegetative biomass. As mechanical isolation of developing embryos is laborious and difficult we are adapting the INTACT nuclear isolation technique (Deal and Henikoff, 2010) to our system. We have developed a construct that tags the nuclear membrane of embryonic cells and allows us to pull down the nuclei for subsequent molecular analysis. We plan to use the DNA of these nuclei to perform ChIP and methylome analyses of the parents and the corresponding hybrids and to compare with the data previously obtained from hybrid seedlings.

POS-TUE-013

EXPLORING THE ROLE OF RNA 5-METHYLCYTOSINE IN *ARABIDOPSIS THALIANA*

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There are over 100 different RNA modifications described in plants and animals and one example is 5-methylcytosine (m^5C). Currently two RNA methyltranferases (RMTases) are known to catalyze m^5C, DNMT2 (DNA Methyltransferase 2) and NSUN2 (NOP2/Sun domain protein 2). *Dnmt2* mutants in *Arabidopsis*, fruit flies and mice appear wild type under standard laboratory conditions however, *dnmt2* mutants in flies are more susceptible to heat and oxidative stress. In mice, *nsun2* mutants are smaller and have cell differentiation defects however almost nothing is known about *nsun2* mutants in plants. We identified two putative NSUN2 homologues, NSUN2A and NUN2B in the *Arabidopsis thaliana* genome and isolated T-DNA insertions in both genes. Of interest NUNS2A appears not to contain the methyl donor domain and may be non-functional. We are further characterizing both *nsun2a* and *nsun2b* single and double mutants. Previously only one m^5C site, C38, has been described in the anticodon loop of tRNA Asp (GUC) of Arabidopsis and requires DNMT2. We identified three new m^5C sites in the variable loop of tRNA Asp (GUC) at positions C47, C48 and C49 and are currently testing if they are dependent on DNMT2 or NSUN2. To further our understanding of m^5C in the transcriptome of *Arabidopsis*, we are undertaking genome sequencing of bisulfite treated RNA from developing siliques one day after pollination. Ongoing work will determine the RMTases required for these specific m^5C sites.

POS-WED-014

MIR396 REPRESSION OF GRFS INHIBITS CELL PROLIFERATION BY UV-B RADIATION IN *ARABIDOPSIS THALIANA* LEAVES

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Because of their sessile lifestyle, plants are continuously exposed to solar UV-B radiation. Inhibition of leaf growth is one of the most consistent responses of plant exposure to UV-B. In this work, we have investigated the role of GROWTH-REGULATING FACTORS (GRFs) and of microRNA miR396 in UV-B-mediated inhibition of leaf growth in *Arabidopsis thaliana* plants. We found that miRNA396 is up-regulated by UV-B radiation in proliferating tissues and that this induction is correlated with a decrease in *GRF1*, *GRF2* and *GRF3* transcripts. Induction of miR396 results in inhibition of cell proliferation, and this outcome is independent of the UV-B photoreceptor UVR8. Transgenic plants expressing an artificial target mimic directed against miRNA396 (*MIM396*) to decrease the endogenous miRNA activity of plants expressing miRNA396-resistant copies of several *GRFs* are less sensitive to this inhibition. Consequently, at intensities that can induce DNA damage in Arabidopsis plants, UV-B inhibits leaf growth by inhibiting cell division in proliferating organs, a process mediated by miR396 and GRFs.

POS-TUE-015

RAPID RECOVERY GENE SILENCING: THE ROLE OF SMALL RNAS AND RNA DECAY IN STRESS MEMORY AND RECOVERY

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Plants have developed sophisticated and intricate mechanisms to avoid and tolerate stresses in their environment. Small RNAs and epigenetic pathways are key regulators of a plant's response to the environment. Adverse conditions can acclimate plants against future environmental challenges, leaving a plant in a primed state with a lingering imprint or memory of the stress. However, memory and acclimation must also be balanced against the need for a rapid recovery and resetting once the stress has dissipated. We are using second-generation RNA sequencing technologies to explore the transcriptome dynamics of high-light and drought stress defense, recovery and memory. In particular, we are investigating the role of small RNAs and RNA decay in a plant's active recovery from stress. High-light stress leads to the rapid up-regulation of many transcripts within minutes. We also observe that stress-responsive transcripts can be equally rapidly silenced again. Some mRNAs are transcribed to a threshold level and then rapidly decayed, while other transcripts are silenced once the stress stimulus is removed. We term these phenomena Rapid Recovery Gene Silencing (RRGS). To investigate the prevalence and mechanisms behind RRGS we are using RNA-seq, small RNA-seq and PARE-seq (degradome analysis) in combination with analysis of Arabidopsis mutants.

POS-WED-016

ALL MICRORNA TARGET SITES ARE EQUAL BUT SOME ARE MORE EQUAL THAN OTHERS: INVESTIGATING THE INFLUENCE OF TARGET SITE CONTEXT ON MICRORNA EFFICACY

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MicroRNAs (miRNAs) direct the silencing of mRNA transcripts bearing complementary 'target' sequences. In animals, it is now well established that diverse contextual features beyond the borders of the target sequence itself can enhance or inhibit the efficacy of a transcript's silencing by a miRNA. This remains an under-explored phenomenon in plants, with miRNA:target complementarity widely considered the only major determinant of miRNA efficacy. The present study utilises the *Arabidopsis* miR159:*MYB33/65* system to address the possibility that there are additional contextual influences at play. MiR159 silences the redundant genes *MYB33* and *MYB65*. The mir159ab mutant exhibits an abnormal phenotype underpinned by the deregulation of *MYB33/65* expression. We have generated four artificial miRNAs (amiRNAs) targeting distinct sequences at different positions throughout the *MYB33/65* transcripts and determined the frequency with which these, as well as endogenous miR159a, could rescue the mir159ab phenotype by silencing *MYB33/65* expression. Each amiRNA shares complementarity with its target site essentially equivalent to the endogenous miR159:*MYB33/65* relationship, yet we observed substantial variations in their efficacies, with no amiRNA operating so effectively as miR159a. This demonstrates that target site context, not just complementarity, has an important influence on miRNA efficacy. Aiming to identify the specific contextual phenomena involved, we next attempt to establish a role for target site accessibility and/or flanking-sequence composition in miRNA efficacy.

POS-TUE-017

MEASURING THE DURATION OF WINTER: IT'S ALL IN THE CHROMATIN

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The promotion of flowering seen in Arabidopsis plants exposed to prolonged periods at low temperatures (winter), is associated with the repression of *FLOWERING LOCUS C* (*FLC*), a repressor of flowering. *FLC* is downregulated at low temperatures and its expression remains low when plants are returned to warmer temperatures. An intriguing aspect of vernalization is the quantitative nature of the response, indicating that plants can measure the duration of winter. The extent of *FLC* repression and the promotion of flowering are correlated with the length of time plants are exposed to vernalizing temperatures. We have previously shown that Polycomb proteins play a role in the quantitative response by regulating the rate of induction of *VERNALIZATION 3* (*VIN3*), which is required for repression of *FLC*. This is only part of the story as it has recently been shown that the permanent switch between the on and off activity states of *FLC* occurs independently in individual cells, and that the number of cells in which *FLC* is switched off increases with time in the cold. As time progresses the average level of *FLC* expression decreases as *FLC* is switched off in more cells. This on/off switch is associated with changes in *FLC* chromatin suggesting that the counting mechanism resides in *FLC* chromatin. We have uncovered the order of events associated with *FLC* repression and propose a mechanism by which the duration of cold is measured.

POS-WED-018

MOBILITY OF PHOSPHATE STARVATION-RESPONSIVE MICRORNAS IN *ARABIDOPSIS THALIANA*

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Plants detect and respond to environmental changes through the regulation of associated genetic pathways. MicroRNAs are important gene regulators for plant development and environmental stress response pathways, and have recently been implicated as phloem-mobile signalling molecules during nutrient starvation conditions. We analysed the expression of phosphate-responsive microRNAs and microRNA*s in an Arabidopsis scion-rootstock graft system. Reciprocal grafts were generated using scion-rootstock combinations of wild-type Col-0 and *hen1* microRNA biosynthesis mutant lines. MicroRNAs synthesised in *hen1* mutants are unstable and are rapidly degraded; thus *hen1* plants contain little to no functional microRNAs. MicroRNA mobility is indicated by the accumulation of specific microRNAs in *hen1* scions or rootstocks of *hen1*-Col-0 graft combinations, showing that they are mobile from Col-0 scions or rootstocks across the graft union. Using this system, we identified that miR399d*, miR827 and miR2111a species are mobile from shoots to roots in Arabidopsis. From comparison of the abundances of microRNAs and their respective microRNA*s, we determined that mobile microRNAs have the capacity for long-distance movement independent of the corresponding microRNA*. We are continuing our study on the mobility of these microRNAs using a microRNA-sensitive fluorescent reporter system for *in planta* visualisation of microRNA expression and movement in Arabidopsis.

POS-TUE-019

A NATURE OF A STRESS-ACTIVATED TRANSPOSON AND THE EFFECT ON THE HOST GENOME

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Eukaryotic genomes consist of a significant extent of retrotransposons that are suppressed by host with epigenetic mechanisms. Although most transposons are silent, certain genomic shocks, such as an environmental stress might trigger transposon activation. In plants, small interfering RNAs (siRNAs) are responsible for RNA-directed DNA methylation (RdDM) that suppresses transposon activities. Recent new findings revealed that siRNAs control not only transcriptional activation, but also suppress transgenerational transposition of a heat-activated transposon. This finding implies a crucial role of the siRNA pathway in restricting retrotransposition triggered by environmental stress. Further, since transposons can affect the regulation mechanisms of host gene, it is likely that transposons have co-evolved as an important factor for plant development and adaptation. We analyzed stress-tolerant mutants that were produced from the transposon-inserted population. Genetic and epigenetic analyses revealed a nature of the stress-activated transposon.

POS-WED-020

A ROLE FOR LONG NON-CODING RNAS IN ARABIDOPSIS SEED DEVELOPMENT?

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Transcriptomic studies from many eukaryotic species have shown that amongst the protein coding mRNA, there is another level of complexity; RNA that appears to have no protein coding potential yet emerging evidence has indicated important biological roles. One class are long non-coding RNAs (lncRNAs) that are considered to be longer than 200 nts in length and have open reading frames that are less than 100 amino acids. In animals, lncRNAs have diverse roles acting in many aspects of gene regulation of which the molecular mechanisms of which are only starting to be uncovered. However in plants only three lncRNAs have been characterised to date. Performing second generation directional Illumina sequencing on ribosomal RNA depleted total RNA from *Arabidopsis thaliana* one day after pollination siliques, 8,105 potential lncRNAs were identified; 1,424 intergenic and 6,681 intronic lncRNAs. Interestingly lncRNAs were identified that are transcribed in both sense and antisense directions within intergenic regions, intronic regions, span exons, introns, UTRs or combinations thereof. LncRNAs associated in endosperm development is being pursued by single cell nuclei purification methodologies, to identify candidates for functional characterisation. Extending upon this, a potential role of lncRNAs in post-fertilisation hybridisation barriers is being explored through inter-species and inter-genus crosses. Our long-term aim is to determine the functional roles of lncRNAs during endosperm development and speciation.

POS-TUE-021

SECONDARY STRUCTURE OF MIR156 PRECURSORS IS IMPORTANT IN AMBIENT TEMPERATURE-RESPONSIVE MIRNA BIOGENESIS IN FLOWERING TIME REGULATION IN *ARABIDOPSIS THALIANA*

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Plant microRNAs (miRNAs) are small non-coding RNAs playing an important regulatory role in plant development. In this study, we found that expression of mature and precursor miR156a and c, which are considered major loci producing miR156, were anti-correlated at different ambient temperatures. RLM 5-RACE assay revealed that more cleavage products from miR156a and miR156c precursors were produced at a low temperature (16°C), suggesting that discrapance in the level of precursor and mature miR156 was caused by enhanced cleavage of miR156 precursor at a low temperature. Based on the comparison of expression level of precursors of ambient temperature-responsive miRNA (ATRM) with those of their mature forms at different ambient temperatures, we could hypothesize that ATRM is divided into two groups, being regulated at transcriptional level and at biogenesis level. The analysis of secondary structure of ATRMs showed some difference between correlated and anti-correlated ATRMs such as burge number and mature miRNA position. Analysis of immino proton spectrum and immino proton exchage velocity by NMR suggested that only one base change located basal part of miR:miR* can change the stability of first cleavage site in miRNA processing. The plants transformed by various mutagenesis constructs in miR156a precursor showed difference in flowering time. These flowering time changes are consistent with differential accumulation of miR156 expression. Taken together, our results suggest that the secondary structure of miR156 precursors is important in ambient temperature-responsive miRNA biogenesis in flowering time regulation in plants.

POS-WED-022

CHARACTERISING MUTANTS IN ROOT-TO-SHOOT MOBILE SILENCING

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RNAi-derived mobile silencing signals have been known for more than a decade in plants and some animals. Proteins that transport RNA silencing signals have been identified in the nematode *Coenorhabditis elegans*, but their counterparts in plants remain a mystery. To explore the factors underlying signal transmission, we performed an EMS screen for *Arabidopsis* mutants deficient in root-to-shoot silencing transport using our dexamethasone-inducible root-to-shoot silencing system (RtSS; Plant Physiology 159: 984-1000). Mutants displayed four types of silencing defect: no silencing; an altered silencing pattern; an increased rate of mobile silencing; a decreased rate of mobile silencing. Plants that showed no silencing were assumed to be deficient in the gene silencing machinery or the dexamethasone-signaling pathway and were not analysed further. We screened 110,000 *Arabidopsis* plants and recovered one mutant (*vasc1*), with a vascular silencing pattern rather than the usual clearly defined advancing front of cell-to-cell silencing through shoot tissues. A second mutant line showed increased movement of silencing (*imos1*), in which analysis of symplastic dye movement showed that leaf plasmodesmata were more open than in RtSS wildtype plants. Two mutants showed root silencing but little or no spread of silencing into the aerial tissue. In these mutants, silencing stopped at the hypocotyl-epicotyl junction which we previously identified as a critical zone for signal transmission. All four mutants were crossed with the parental RtSS line and the F1 progeny selfed; DNA from at least 50 individual F2 progeny was pooled for deep sequencing followed by SHOREmap analysis to identify the causal mutations. Identification of these genes may finally reveal components of mobile silencing transport in plants.

POS-TUE-023

TARGETED ANALYSIS OF PARENT-OF-ORIGIN DEPENDENT ALLELIC EXPRESSION IN ARABIDOPSIS ENDOSPERM

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Genomic imprinting is responsible for the differential expression of alleles depending on their parent-of-origin. In flowering plants imprinting primarily occurs in the endosperm, a triploid fertilisation product that acts to support the developing embryo. Here, we discovered 24 new imprinted genes in Arabidopsis using High Resolution Melt analysis and next-generation sequencing. Using these genes, along with published imprinted genes, we explored differences between maternally expressed genes (MEGs) and paternally expressed genes (PEGs). This revealed that as a group the PEGs are predominantly expressed in the endosperm during the early proliferative stage of development, whereas the MEGs have a more general expression across seed development. The MEGs and PEGs also differed in their regulation and function, with the PEGs being overrepresented in genes involved in cell cycle and DNA conformation. These results provide the first evidence that PEGs play a specific role during endosperm proliferation. As imprinting is predicted to underlie the parent-of-origin effects that lead to altered endosperm proliferation during interploidy crosses, we also analysed the influence of altered genomic dosage. We found that interploidy crosses cause complex alterations in allelic expression, including imprint breakdown. Overall, this work advances our understanding of the evolutionary roles and mechanisms by which imprinting influences seed development.

POS-WED-024

RNA STRUCTURE CLUSTERING: A PATH TO FIND NOVEL GENETIC AND EPIGENETIC REGULATORY MODULES

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RNA secondary structure can influence gene expression and protein activity. Some RNA structural elements control process such as transcription, splicing, protein translation and RNA-mediated gene silencing. Most studies investigating non-coding RNAs have focused on microRNA (miRNA) identification and validation. We are developing a bioinformatics pipeline to identify novel RNA classes based on structure, sequence, function and evolutionary conservation. This pilot study only consists of sequences from the Arabidopsis genome. We clustered the 5' messenger RNA (mRNA), untranslated leader regions (UTR) and identified miRNA as well as riboswitch-like RNA structural modules. The evolutionary and biological significance of these RNA modules are under investigation. Our preliminary results reveal that genes harbouring RNA riboswitch-like modules in the 5'UTR are enriched in metabolic processes controlling cellular metabolism.

POS-TUE-025

ENDOGENE PROTECTION FROM RNA SILENCING

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In plants, transgenes are vulnerable to sporadic triggering of RNA silencing through the production of double-stranded RNA (dsRNA) by *RNA-DEPENDENT RNA POLYMERASE 6, RDR6. RDR6* activity is predictably recruited to transgenic but not endogenous transcripts when silencing is initiated by an homologous inverted repeat transgene. This is evidenced by the production of short interfering RNAs (siRNAs) with homology to regions outside of the initiating dsRNA, a process termed transitivity. Transitivity involves a reiterative amplification of the silencing signal which facilitates the systemic spread of transgene silencing. To better understand the vulnerability of transgenes to silencing, *CHALCONE SYNTHASE (CHS)* was chosen as a suitable endogenous reporter to study the mechanisms responsible for an apparent protection of endogenous transcripts from *RDR6* activity in *Arabidopsis thaliana*. A fluorescence reporter system was developed to report on the presence of transitivity in silencing this locus and a number of enhanced silencing lines have been isolated from a mutant screen with this system.

POS-WED-026

TRIPOGON LOLIIFORMIS miRNAome: THE ROLE OF microRNAs IN STRESS RESPONSE IN RESURRECTION PLANTS

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Plant microRNAs (miRNAs) are small endogenous non-coding RNAs involved in post-transcriptional regulatory mechanisms in growth, development and in response to biotic and abiotic stress. To date, extensive studies on the role of miRNAs in plants adaptive responses to abiotic stresses have been limited to model plant species and a few economically important crops. There exists limited information on the role of miRNAs in response to desiccation in extremophiles such as resurrection plants. *Tripogon loliiformis* is a native Australian resurrection grass that can withstand extreme drought or desiccation to levels below 5% Relative Water Content (RWC) and survive in the air-dry state for months to several years with the ability to regain normal function in 24 - 72 hours upon rewatering. Next generation sequencing of *T. loliiformis* small RNAs from the roots and shoots of plants subjected to dehydration stress to levels below 40 % RWC resulted in identification of 145 conserved and 91 predicted novel miRNAs. The identified conserved miRNAs included a total of 50 multi-member families with top hits from monocots such as sorghum, rice, and maize. Expression analysis showed a significant number of miRNAs were highly expressed during dehydration majority of them in the shoots. Resurrection plants employ unique desiccation tolerance mechanisms that enable them to withstand extreme water deficit. Preliminary data analysis suggests that miRNAs plays a crucial regulatory role in resurrection plants adaptive response to extreme dehydration stress. Following in planta functional studies, these results may be utilised for the generation of genetically enhanced crop varieties.

POS-TUE-027

QUANTITATIVE PROTEOMIC ANALYSIS OF *ARABIDOPSIS THALIANA* DOUBLE-STRANDED RNA BINDING2 (DRB2) KNOCKOUT MUTANT PLANTS

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In *Arabidopsis thaliana*, structure of double-stranded RNA (dsRNA) precursors is predicted to direct recognition by dsRNA binding (DRB) protein family members that form functional partnerships with DICER-LIKE (DCL) proteins for precursor processing into small RNAs (sRNAs). We recently demonstrated that DRB2 is both synergistic and antagonistic to DRB1 in microRNA biogenesis in *Arabidopsis* meristematic tissue. Specialized, redundant and antagonistic functions for DRB2 and DRB4 have also been shown. DRB2 is involved in several sRNA pathways and its function and targets are not yet fully known. We are therefore analysing the proteome of drb2 mutant plants to identify proteins that are regulated by DRB2. Metabolic labelling of drb2 and wild-type (Col-0) plants was performed by growing plants on MS media with stable nitrogen isotope ^{15}N ($K^{15}NO_3$ and $^{15}NH_4{}^{15}NO_3$). Three week old plants grown on ^{15}N and ^{14}N-enriched media were pooled and total protein extracted. Each pool was made at a fresh weight ratio of 5:1 (drb2 to Col-0). Proteins were separated by SDS-PAGE and the stained gels sectioned into 20 pieces and digested with trypsin. Peptides were analysed by mass spectrometry and proteins were identified. The spectra of $^{15}N/^{14}N$ peptide pairs were extracted and the relative amounts calculated. Proteins present at different levels in drb2 knockout mutant plants are currently under analysis.

POS-TUE-027A

GENOME-WIDE DISTRIBUTION OF ARABIDOPSIS LINKER HISTONES IN PLANTS GROWN UNDER NORMAL AND LIMITED LIGHT CONDITIONS

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Eukaryotic genome is packaged in the nucleus into a highly compact nucleoprotein complex – the chromatin. In a basic chromatin unit, the nucleosome, DNA is wrapped around the octamer of the four core histone proteins. The linker histone (H1) binds to DNA entering and exiting the nucleosomal core particle and has an important role in establishing and maintaining higher order chromatin structures. The linker histones are conserved and ubiquitous structural components of eukaryotic chromatin. In both animals and plants co-exist multiple non-allelic variants of H1 which differ in DNA/nucleosome binding properties and have been implicated in control of genetic programs during development and differentiation. Plants possess an evolutionary conserved branch of “stress inducible” variants of H1. In Arabidopsis occur two main linker histone variants (H1.1, H1.2) and the stress-inducible variant H1.3. While the genome-wide occupancy patterns of core-nucleosome histones are relatively well known, our knowledge of the preferences for H1 histone binding across the genome is limited. Taking advantage of the Arabidopsis lines expressing GFP-tagged histones H1.1, H1.2 and H1.3 and performing ChIP-on-Chip analysis using GFP antibody, we elucidated the genome-wide binding patterns of all three Arabidopsis H1 variants, before and after low-light stress.

POS-WED-028

INVESTIGATING THE SYNERGY BETWEEN RIBONUCLEASES AND PENTATRICOPEPTIDE REPEAT PROTEINS WITHIN HIGHER PLANT CHLOROPLASTS

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The endosymbiotic uptake of a cyanobacterium by a primitive eukaryote gave rise to the chloroplast, which performs vital cellular functions such as photosynthesis. The prokaryotic ancestry of chloroplasts is particularly evident by the organisation of genes into operons, eubacterial translation and transcription machinery and the ribonucleases involved in RNA metabolism. The regulation of chloroplast gene expression has levels of complexity not found in prokaryotes, particularly post-transcriptional processes including processing of polycistronic transcripts, RNA editing and intron splicing. These events are facilitated by nucleus-encoded endo- and exo-ribonucleases, along with RNA-binding proteins such as pentatricopeptide repeat (PPR) proteins. PPR proteins have massively expanded in higher plants and 450 of them exist in Arabidopsis. Two main ribonucleases involved in these processes are RNase E and RNase J and the importance of their nuclease function is demonstrated by their mutant phenotypes. Arabidopsis RNase E null mutants are chlorotic and display stunted growth, whereas, RNase J null mutants prevent embryo development. Chloroplastic RNase J exhibits both exo- and endo-nuclease activities, which play an important role in 5' end maturation and is directed by PPR proteins. Unlike the E. coli ortholog, chloroplast RNase E appears not to function in 5' end maturation, however, recent reports have concluded this enzyme is responsible for intercistronic cleavages. We have shown polycistronic transcripts in RNase E null plant material are appropriately processed. This suggests that functional overlap with RNase J maybe occurring. To further understand the role of RNase E in chloroplast RNA metabolism, we have performed strand-specific RNA sequencing to define the chloroplast transcriptome in the null mutant. An update on the progress of this research will be presented.

POS-TUE-029

POLY(A) SPECIFIC RIBONUCLEASES, ATCCR4S ARE IMPORTANT FOR THE STARCH METABOLISM

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Starch is important for plant growth and development as energy source derived from photosynthesis. Here, we presented the involvement of mRNA turnover control in starch metabolism. Removal of poly(A) tail is the first and rate-limiting step of mRNA turnover for many transcripts in eukaryotes. Carbon Catabolite Repressor 4 (Ccr4) has been identified as the major cytoplasmic deadenylase in yeast. Arabidopsis has homologs of the yeast Ccr4 consisting of six members. Among them, AtCCR4-1 and AtCCR4-2 are most similar to yeast Ccr4. FLAG-tagged AtCCR4-1 or AtCCR4-2 exhibited poly(A) specific degrading activity in vitro. Transient expression analysis using the GFP fusion of AtCCR4-1 or AtCCR4-2 indicated that both are localized in specific granule called P-body, which is the region within the cytoplasm consisting of many enzymes involved in mRNA turnover. To elucidate the functional significance of AtCCR4-1 and AtCCR4-2 in vivo, the double mutants were constructed. The double mutants showed the insensitivity to a high level of sucrose. Levels of sucrose were reduced and these of leaf starch were likely to be increased in double mutants, although no difference was observed in the glucose level. In addition, global gene expression profiling between double mutant and wild-type plants by microarray revealed that one of the up-regulated genes in the double mutants is involved in the starch biosynthesis, suggesting the possible involvement of AtCCR4 in regulating the gene expression related to starch metabolism.

POS-WED-030

TRANSCRIPTOME ANALYSIS OF ARABIDOPSIS HYBRIDS DURING EARLY EMBRYO DEVELOPMENT

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In many species, hybrid progeny of selected parental lines have enhanced performance relative to their parents. This phenomenon is known as hybrid vigour or heterosis. Although heterosis has been used extensively by breeders to increase the performance of crop plants, our understanding of its molecular basis is still rudimentary. Heterosis is not a single end point character but a summation of responses in different tissues and at various stages of development. It almost certainly begins in the single cell zygote with the interaction between the genomes and epigenomes of the two parents occurring after the sperm cell fuses with the egg cell in the embryo sac. The interactions of these two sets of instructions result in the unique characteristics of the hybrid. Our lab is currently using hybrids between Arabidopsis accessions as a model for dissecting the genetic and epigenetic contributions of hybrid heterosis. In particular we are interested in determining the control mechanisms behind heterosis during early development. To investigate this, we are studying the reciprocal hybrids generated from Arabidopsis ecotypes C24 and Landsberg erecta that show different degrees of heterosis in those early stages of development. To identify genes involved in early hybrid vigour, we have performed a transcriptomic analysis of the reciprocal hybrids and their parents during seed development. We have isolated RNA from developing embryos at globular stage and heart stage and performed transcriptome analyses. An update of our results will be presented.

POS-TUE-031

PSROBOT: A WEB-BASED PLANT SMALL RNA META-ANALYSIS TOOLBOX

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Small RNAs (smRNAs) in plants, mainly microRNAs and small interfering RNAs, play important roles in both transcriptional and post-transcriptional gene regulation. The broad application of highthroughput sequencing technology has made routinely generation of bulk smRNA sequences in laboratories possible, thus has significantly increased the need for batch analysis tools. PsRobot is a web-based easy-to-use tool dedicated to the identification of smRNAs with stem-loop shaped precursors (such as microRNAs and short hairpin RNAs) and their target genes/transcripts. It performs fast analysis to identify smRNAs with stem-loop shaped precursors among batch input data and predicts their targets using a modified Smith-Waterman algorithm. PsRobot integrates the expression data of smRNAs in major plant smRNA biogenesis gene mutants and smRNA-associated protein complexes to give clues to the smRNA generation and functional processes. Besides improved specificity, the reliability of smRNA target prediction results can also be evaluated by mRNA cleavage (degradome) data. The cross species conservation statuses and the multiplicity of smRNA target sites are also provided. PsRobot is freely accessible at **http:// omicslab.genetics.ac.cn/psRobot/**.

POS-WED-032

WIDE-SPREAD LONG NON-CODING RNAs (lncRNAs) AS ENDOGENOUS TARGET MIMICS (eTMs) FOR microRNAs IN PLANTS

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Target mimicry is a recently identified regulatory mechanism for microRNA functions in plants in which the decoy RNAs bind to microRNAs via sequence complementary therefore to block the interaction between microRNAs and their authentic targets. Both endogenous decoy RNAs (microRNA target mimics) and engineered artificial RNAs can induce target mimicry effects. Yet till now, only the IPS1 (Induced by Phosphate Starvation 1) RNA has been proven to be functional endogenous microRNA target mimics (eTMs). In this work, we developed a computational method and systematically identified intergenic or non-coding gene-originated eTMs for 20 conserved microRNAs in Arabidopsis and rice. The predicted microRNA binding sites were well conserved among eTMs of the same microRNA, whereas sequences outside of the binding sites varied a lot. We proved the eTMs of miR160 and miR166 as functional target mimics and identified their roles in the regulation of plant development. The effectiveness of eTMs for other three miRNAs was also confirmed by transient agroinfiltration assay.

POS-TUE-033

PHOSPHORYLATION OF UPF1 IS ESSENTIAL FOR NMD IN *ARABIDOPSIS*

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NMD (Nonsense-Mediated Decay) is an RNA quality control process, which removes mRNAs containing premature termination codons located within protein-coding regions. Despite intense research in recent years dedicated to understanding NMD mechanisms in many different organisms, information regarding NMD complex formation in plants is still very incomplete. The SMG1 PIKK kinase-mediated phosphorylation of UPF1 N- and C- terminus is a critical step for NMD in mammals and C. elegans. Phospho-UPF1 is bound by 14-3-3 like proteins (SMG5-7 heterodimer and/or SMG6), and then these proteins trigger transcript decay and dephosphorylation of UPF1. Although SMG1 kinase homologs are not present in plants, several sources of evidence suggest that UPF1 phosphorylation is also important for plant NMD. The conserved S/TQ phosphorylation motifs are present in plant UPF1. Previously, it has been demonstrated that the 14-3-3-like potential phosphoserine, phosphothreonine binding domain of AtSMG7 is required for plant NMD. Moreover, it has been shown that the N-terminal region of AtUPF1 is phosphorylated. To clarify whether UPF1 phosphorylation is essential for plant NMD, we performed detailed functional analyses of several AtUPF1 mutants using the transient NMD assay in *Nicotiana benthamiana* combined with virus-induced gene silencing (VIGS). Moreover, the in vivo phosphorylated AtUPF1 residues were identified by mass spectrometry from transiently expressed protein as well as precipitated from stably transformed *Arabidopsis thaliana*. Our results indicates that AtUPF1 is phosphorylated at multiple sites, however only some, conserved residues are required for NMD.

POS-WED-034

VISUALIZATION OF MRNAS *IN VIVO* IN *ARABIDOPSIS* USING THE MODIFIED MS2 SYSTEM

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The localization and distribution of an mRNA can play an important role in controlling its translation both spatially and temporally. mRNAs are assembled into granules by association with a wide range of RNA binding proteins (RBP). These granules can be transported to specific cytoplasmic destinations or be associated with processing or storage of mRNAs and hence regulate the activity of the mRNA. We are using aptamer-tagging systems (Schonberger et al., 2012) to visualize RNA in plant cells in vivo. The fluorescence-tagged coat protein of phage MS2 (MSCP) binds a specific RNA stem-loop, MS2. Similarly the fluorescent-tagged λN22 binds a specific RNA stem loop, Box b. In mammalian cells, MS2 and λN22 systems have been widely used to study mRNAs tagged with MS2 or Box b. In plant cells, however, there has been limited application of these two systems to localize mRNA. We made a single construct containing MSCP-GFP binding protein and MS2-tagging cassette in a single vector. In the presence of a tagged PROFILIN mRNA expressed from the 35S promoter, GFP fluorescence was observed in the cytoplasm of transiently transformed N. benthaniana leaf cells and stably transformed Arabidopsis root cells. We also expressed the tagged PROFILIN mRNA from its own promoter and have also observed GFP fluorescence in the cytoplasm of transformed cells, but at a lower level than that when using the 35s promoter. Therefore we are able to use the MS2 system to visualise RNAs in both transiently and in transgenic plants. We are now using the system to characterise mRNAs expressed at various stages of seed development to understand how post-transcriptional regulation contributes to the expression of these genes. Reference: Schonberger J, Hammes UZ and Dresselhaus T. (2012) In vivo visualization of RNA in plants cells using the λN22 system and a GATEWAY-compatible vector series for candidate RNAs. The Plant Journal, 71(1), 173-81. doi: 10.1111/j.1365-313X.2012.04923.x.

POS-TUE-035

PLANT MICRORNAS DISPLAY DIFFERENTIAL 3'-TRUNCATION AND TAILING, MODIFICATIONS WHICH ARE ARGONAUTE1-DEPENDENT AND CONSERVED ACROSS SPECIES

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Plant small RNAs are 3' methylated by the methyltransferase HEN1. In plant hen1 mutants, 3' modifications of small RNAs, including oligo-uridylation ("tailing"), are associated with accelerated degradation of miRNAs. By sequencing small RNAs of wildtype and hen1 mutants from Arabidopsis, rice and maize, we found 3' truncation prior to tailing is widespread in these mutants. Moreover, the patterns of miRNA truncation and tailing differ substantially among miRNA families but are conserved across species. The same patterns are also observable in wildtype libraries from a broad range of species, only at lower abundances. ARGONAUTE (AGO1), even with defective slicer activity, can bind these truncated and tailed variants of miRNAs. An *ago1* mutation in *hen1* suppressed such 3' modifications, indicating that they occur while miRNAs are in association with AGO1, either during or after RISC assembly. Our results showed AGO1-bound miRNAs are actively 3' truncated and tailed, possibly reflecting the activity of co-factors acting in conserved patterns in miRNA degradation.

POS-WED-036

DEVELOPMENT OF PAVEMENT CELL SHAPE IN ARABIDOPSIS COTYLEDONS

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Arabidopsis epidermal pavement cells develop into irregular complex shapes with undulating lobes. Previous studies have made inferences about the growth of these cells from images taken at only a single time point. Here, pavement cell development was characterised from 3 to 5 days after sowing to investigate how lobes grow. A new method to apply fluorescent landmarks on the cell surface allowed heterogonic expansion of the outer periclinal wall and anticlinal wall to be measured. Detailed assessment of the relative expansion in a contiguous region of cells and at individual lobes revealed that a unique pattern of heterogonic expansion in the outer periclinal surface can explain how lobes form. Actin in GFP-fABD2 plants and microtubules in GFP-TUB6 plants were imaged over time to explore the role of the cytoskeleton in lobe formation. Cell growth and cytoskeleton arrangements were followed after application of the cytoskeletal disrupting drugs cytochalasin D and oryzalin. Surprisingly, while microtubules showed persistent enrichment at sites where lobes formed, the development of pavement cell shape was not altered by disruption with oryzalin. In contrast, actin showed little persistence at sites where lobes formed but, on disruption with cytochalasin D, formed foci at developing lobes while cell growth and lobe formation were inhibited. Analysis of pavement cell growth showed areas of restricted expansion correlated to patterns of microtubules. In contrast, actin appeared to facilitate overall growth of cells.

POS-TUE-037

IDENTIFICATION OF *ATMYB80* DOWNSTREAM TARGET GENES INVOLVED IN POLLEN DEVELOPMENT USING CHIP

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The Arabidopsis *AtMYB80* gene is a member of the R2R3 MYB gene family whose expression is restricted to the tapetum of developing anthers and trichomes. Downregulation of the *AtMYB80* gene leads to abnormal tapetum and pollen development. Pollen grains are distorted in shape and have reduced cytoplasmic content. Furthermore, blocking *AtMYB80* gene expression results in complete male sterility and failure to set seed in Arabidopsis plants. However, little is known about the AtMYB80 pathway or the genes it regulates. The ChIP (Chromatin Immunoprecipitaion) assay allows for protein-DNA interactions to be cross-linked to the DNA they are binding to and immunoprecipitated with an antibody specific to the protein of interest. To identify putative downstream target genes of AtMYB80, ChIP was performed on 13 genes. These genes were selected based on microarray results which compared differential gene expression in wildtype vs *atmy80* mutant anthers in an inducible mutant background. Two genes were found to be direct targets and were characterized further. T-DNA insertion mutants of the two genes were analysed and scanning electron microscopy (SEM) was used to examine pollen. SEM micrographs show severe disruption to pollen development with the lack of well developed pollen grains. Alexander staining of anthers also showed pollen grains which were collapsed and irregularly shaped with an abundance of cellular debris when compared to the wildtype in both insertion mutants.

POS-WED-038

EMF1 REGULATES VERNALIZATION SIGNALING BY THE REPRESSION OF *VIN3*

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The suppression of floral repressor *FLOWERING LOCUS C (FLC)* is a crucial mechanism for the vernalization-dependent promotion of flowering event. *VERNALIZATION INSENSITIVE 3 (VIN3)* encoding a PHD finger domain protein is associated with the epigenetic repression of *FLC*. The induction of *VIN3* is one of the earliest responses by vernalization. However, it is unclear how plants sense the winter signal. One way to reveal the question is to isolate upstream components of *VIN3*. To search the *VIN3* upstream components, we generated a transgenic line with the promoter of *VIN3* fused to GUS reporter gene and performed *Agrobacterium*-mediated activation tagging mutagenesis. As a result, these transgenic plants exhibited vernalization-induced GUS expression. Currently, we have isolated a vernalization hyposensitive mutant *X79* which showed decreased expression of *VIN3* during vernalization. In *X79* mutant, both GUS signal and *VIN3* transcript were reduced compared to WT. *X79* contains a T-DNA at the promoter region of *EMBRYONIC FLOWER 1 (EMF1)*, and *EMF1* is overexpressed in *X79*. EMF1 cooperates with PRC2 and binds around the transcription start sites of target genes. It represses the transcription of several *MADS-box* genes and flower organ identity genes. *X79* had a similar induction pattern of *VIN3* in the time course of vernalization treatment but, the level of transcription was reduced compared to WT. In contrast, *VIN3* was de-repressed in *EMF1* loss-of-function mutants at warm temperature. Based on the results, we suggest that *EMF1* suppresses *VIN3* expression at warm temperature and generates specific slow induction of *VIN3* during vernalization.

POS-TUE-039

WDR55 INTERACTS WITH DDB1 AND IS REQUIRED FOR APICAL PATTERNING IN THE ARABIDOPSIS EMBRYO

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Plant reproduction and the development of a seed require coordinated regulation of genes and gene products in gametophytes as well as in the different organisms of the seed. Protein ubiquitination by cullin (CUL)-RING E3 ligases (CRLs) regulates an extensive range of biological processes by attachment of ubiquitin to substrate proteins to either promote their degradation by the UBIQUTIN-26S proteasome pathway, or by changing their function or chromatin context. CRL4 ligases were recently shown to exert their specificity through the binding of various substrate receptors, which bind the CUL4 interactor DDB1 through a DWD or a WDxR motif. In a segregation-based mutagenesis screen we identified a WDxR motif-containing protein (WDR55) required for male and female gametogenesis and seed development. We demonstrate that WDR55 physically interact with DDB1A in planta, suggesting WDR55 to be a novel substrate recruiter in CRL4 ubiquitin ligase complexes. Examination of the mutant allele *wdr55-1* revealed a delay in the fusion of the polar nuclei in embryo sac development, in addition to embryo and endosperm developmental arrest. Interestingly, the observed embryo and endosperm phenotype is reminiscent to CUL4 and DDB1A/B loss of function, in support of a regulatory role of a putative $CUL4^{WDR55}$ ligase complex. *wdr55-2* embryos suggest a defect in the transition to bilateral symmetry in the apical embryo domain. Auxin distribution in the *wdr55-2* embryo by means of the synthetic *DR5* reporter appears not to be affected. However, the lack of bilateral symmetry and further localization failure of DORNROESCHEN, a direct target of the auxin response factor protein MONOPTEROS, may suggest a WDR55 function in targeting genetic components regulated by auxin. Currently, we have isolated a homozygous *WDR55* knockout, and here we report that the adult plants display pleiotropic phenotype characteristics of which many are reminiscent of mutants in auxin regulated pathways.

POS-WED-040

SHORT INTERNODES/STYLISH GENE FAMILY MEMBERS ARE IMPORTANT FOR DEVELOPMENT OF VARIOUS ORGANS IN *ARABIDOPSIS THALIANA*

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The *SHI/STY* transcription factors act highly redundantly in the regulation of organ development, e.g. patterning of the gynoecium, stamen identity and leaf polarity (Kuusk et al., 2006; Staldal et al., 2008; Staldal et al., 2009), and exert some of their functions by regulating the auxin biosynthesis gene *YUC4* (Sohlberg et al., 2006; Eklund et al., 2010). Here we will present data showing that the *SHI/STY* genes, in addition to regulating the developmental processes mentioned above, also are important for cotyledon and leaf vascular development (Baylis et al., 2013). In addition, STY1 regulates the expression of many other genes, including a subset that encodes proteins affecting cell wall loosening or cell wall composition. Preliminary data concerning the cell wall related genes will be presented.

POS-TUE-041

IDENTIFICATION OF NOVEL COMPONENTS OF THE GENETIC PATHWAYS CONTROLLING SHOOT BRANCH OUTGROWTH

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Shoot branching patterns are critical determinants of the aerial plant architecture. These patterns are elaborated from axillary meristems (AMs) formed in the axils of leaves where they either remain dormant or develop into branches. Long-range signalling promoting bud arrest is controlled by auxin, produced in the shoot apex and transported basipetally, and strigolactones (SL) transported acropetally. In Arabidopsis, SL synthesis and signaling is regulated by the *MAX* genes. Downstream of the SL pathway is *BRANCHED1*, gene encoding a TCP factor that acts inside the buds as a key integrator of signals controlling bud outgrowth. Additional genes acting in the SL pathway and downstream of *BRC1* remain to be identified. To isolate new components of this genetic network we carried out an EMS mutagenesis and screened for mutants with increased branching at high planting density. The mutants isolated were termed *seto* mutants. We initiated the characterization of three (*seto2, seto3 and seto5*). We made F2 mapping populations by crosses with Landsberg erecta wild-type plants. Mapping data in combination with complementation tests confirmed that *seto2* is a *MAX2* allele. *seto3* maps within a region encompassing the *MAX3* locus. Finally *seto5* mapped within a region where no *max* mutants had been characterized. Whole genome sequence of *seto5* individuals helped us find a likely candidate gene. Molecular and functional characterization of this gene will be presented.

POS-WED-042

IDENTIFICATION AND NEXT GENERATION MAPPING OF AN ARABIDOPSIS EMS MUTANT THAT DISPLAYS LENGTHENED PLASTOCHRON AND PLEIOTROPIC EFFECTS ON REPRODUCTIVE ORGANS

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A characteristic feature of plants post-embryonic growth is the initiation of leaf primordia from the shoot apical meristem (SAM) at regular time intervals. The time between the initiation of two successive leaf primordia from the SAM is referred to as the plastochron. Although vital in influencing overall shoot architecture, our current understanding of the underlying genetics of plastochron regulation is very limited. Here, we describe the identification of an *Arabidopsis* mutant, *pla* that affects plastochron index by lengthening the time taken for successive leaves to appear. Accordingly, at different stages of vegetative growth, the *pla* mutant consistently displays reduced number of leaves compared to wild-type plants. In addition, the mutant also displays pleiotropic phenotypes affecting the sizes of floral organs, seeds and cotyledons. Analysis of a segregating mapping population suggests that the phenotypes observed for *pla* are inherited as a single recessive locus. To positionally map the *pla* mutation, an F2 mapping population was generated by crossing *pla* (Col-0) to a polymorphic parent, *Landsberg erecta*. Traditional map-based cloning using polymorphic genetic markers as well as next-generation sequencing was used to identify mutations in three genes that are likely candidates for the *pla* phenotype. Currently, work is being carried out to transform the mutant with the each of the three genes with aim that one of the genomic sequences will be able to complement the lengthened plastochoron phenotype.

POS-TUE-043

REGULATION OF THE EXPRESSION OF *VND7* GENE ENCODING A MASTER REGULATOR FOR XYLEM VESSEL FORMATION BY A GATA TYPE TRANSCRIPTION FACTOR

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In our previous work, *VASCULAR-RELATED NAC-DOMIN7* (*VND7*) encoding a NAC-domain transcription factor was shown to be expressed preferentially in xylem vessel precursors and was indicated to act as a master regulator for xylem vessel formation. However, regulatory mechanisms underlying the expression of *VND7* gene is still largely unknown. Hence, in this study we aimed to identify transcription factors (TFs) that regulate the *VND7* expression. First, we selected TFs with the up-regulated expression during vessel formation based on our microarray data. Next we performed the particle bombardment-based transient assays to test whether each candidate driven by the CaMV35S promoter has a potential to induce *VND7* promoter activity using the *VND7pro*:*Luciferase* construct as a reporter. As a result, we found several TF genes that can activate the reporter expression. Further analysis revealed that two genes encoding GATA type transcription factors, *GATA5* and *GATA12*, are specifically expressed in the central cylinder of the root including the vessel precursors. Moreover, overexpression of *GATA12* resulted in the ectopic formation of vessel-like cells, suggesting a close association of *GATA12* with xylem vessel formation.

POS-WED-044

ATNHX5 AND *ATNHX6* ARE REQUIRED FOR LATERAL ROOT DEVELOPMENT

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AtNHX5 and *AtNHX6* are sodium hydrogen anti-porter proteins localized to the trans golgi network that transport a sodium or potassium ion in exchange for a hydrogen ion across a membrane. They act redundantly and are members of a larger family of NHX proteins that in Arabidopsis thaliana includes *AtNHX1-4*, *AtSOS1* and *AtNHX7*. Like their well characterised relatives *AtNHX1* and *AtSOS1* they are important determinants of salt tolerance. Unlike other members of the NHX family there are conserved orthologs in both yeast and mammals and all of the orthologs have an important role in sub-cellular protein trafficking. The *nhx5 nhx6* double mutant has a severe phenotype and displays slower growth, delayed flowering and inhibited primary root growth. Further investigation revealed a reduction in the number of emerged lateral roots in the *nhx5 nhx6* double mutant due to a distinct defect in lateral root primordia (LRP) development. Analysis of LRP by live imaging, fixed radial sections and staining with the lipophillic membrane dye FM5-95 revealed severe disruptions to cell division, patterning and organization. In wild type plants, a clearly defined auxin gradient with a maxim at the tip of the LRP is required for coordinated cell division and patterning. Visualization of auxin maxim and auxin transport using a DR5:GFP reporter in roots of the *nhx5 nhx6* double mutant revealed that cell to cell auxin transport and the establishment of the auxin gradient are impaired. The constitutive endocytosis and recycling of PIN auxin efflux proteins is essential for directional auxin transport and the establishment of auxin gradients. It is possible that in the *nhx5 nhx6* double mutant the trafficking of PIN proteins is inhibited disrupting auxin gradients and LRP development.

POS-TUE-045

TRANSCRIPTIONAL REGULATION OF THE MEDICAGO *SOC1* GENE FAMILY FOR CONTROLLING FLOWERING

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In Arabidopsis, *FLOWERING LOCUS T* (*FT*) expression is regulated by vernalisation and long days (LD), to induce flowering. FT protein is a mobile flowering signal, which moves from the leaves to the shoot apex, to interact with FLOWERING D (FD) and in turn activate key floral genes, such as *SOC1*, to promote flowering. *SOC1* is also regulated by vernalisation and GA_3 in the absence of LD. Like Arabidopsis, the model legume *Medicago truncatula* is a vernalisation-responsive, long-day plant. In Medicago there are five *FT* and three *SOC1* genes. FTa1 is required to promote flowering, yet the potential role of the *SOC1* genes for flowering time control remains unknown. In Medicago, *FTa1* expression is up-regulated after vernalisation and by LD to promote flowering. Medicago *fta1-1* mutants do not respond to vernalisation and flower late. During a developmental time course study in vernalised wild type plants under inductive LD conditions, an increase in expression of *SOC1b*, and to a lesser extent *SOC1c*, was observed, coinciding with that of *FTa1*. Under the same conditions in *fta1-1* plants, *SOC1b* and *SOC1c* expression is greatly diminished, indicating that expression of *FTa1* is required for *SOC1b* and/or *SOC1c* induction. To further investigate downstream targets of FTa1, we are analysing the transcriptome of Medicago leaves in which *FTa1* has been transiently over-expressed by *Agrobacterium* infiltration, through qRT-PCR and RNAseq analyses.

POS-WED-046

TWO MADS-BOX GENES PREPONDERANTLY EXPRESSED IN ROOT, *XAANTAL1* AND *2* (*AGL12* AND *AGL14*) PARTICIPATE IN FLOWERING TRANSITION

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A large number of genes participate in the regulatory network involve in flowering transition. However, little is known about the proteins that act as activators and at the same time are negative feedback regulators of other genes implicate in this process. We present data for two MADS-box genes *XAANTAL1* (*XAL1*/*AGL12*) and *XAL2*/*AGL14* that are known to be preferentially express in the root, and here we show by in situ hybridization that they also accumulate in the flower meristem. Furthermore, *xal1* mutant alleles are late flowering under long day (LD) photoperiod, while *xal2* alleles are affected particularly under short day (SD). However only XAL2 is sufficient to induce flowering transition, independently of the photoperiod. Genetic and RT-PCR studies showed that under LD, XAL1 regulates *FT* in a partially independent pathway of CO and that XAL2 participate in the up-regulation of *LFY* and *AP1* in a SOC-partially dependent way. Interestingly, under this condition XAL1 represses *XAL2*, while XAL2 is a negative a regulator of *XAL1*, *SOC1* and *AGL24*. Therefore, *XAL1* and *XAL2* formed part of the gene regulatory network that participate in flowering transition and we propose that negative-feedback regulation could give fine-tuning of the network in response to different flowering signals.

POS-TUE-047

CONTROL OF LEAF VEIN FORMATION BY AUXIN SIGNALING

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The beautiful and diverse patterns of veins in plant leaves have fascinated biologists for centuries. In developing leaves, files of cells that will differentiate into veins are selected from within a population of cells of similar shape and size. Molecular details of the selection process are scarce, but available evidence suggests that the process terminates with activation of expression of the HOMEODOMAIN-LEUCINE ZIPPER III transcription factor ARABIDOPSIS THALIANA HOMEOBOX8 (ATHB8) by the AUXIN RESPONSE FACTOR MONOPTEROS (MP). We will present and discuss new evidence which defines more precisely the role of MP-dependent auxin signaling and ATHB8-dependent gene transcription in leaf vein formation.

POS-WED-048

ACCELERATION OF FLOWERING BY CAPE VERDE ISLANDS ALLELES OF *FLOWERING H* IS DEPENDENT ON THE FLORAL PROMOTER FD

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Flowering time in the model plant *Arabidopsis thaliana* is regulated by both external environmental signals and internal developmental pathways. Natural variation at the *FLOWERING H* (*FLH*) locus has previously been described, with alleles present in the Cape Verde Islands accession causing early flowering, particularly after vernalization. The mechanism of *FLH*-induced early flowering is not understood. Here the integration of *FLH* activity into the known flowering time pathways is described using molecular and genetic approaches. The identification of molecular markers that co-segregated with the *FLH* locus allowed the generation of multiple combinations of *FLH* alleles with mutations in flowering time genes in different flowering pathways. Combining an early flowering *FLH* allele with mutations in vernalization pathway genes that regulate *FLC* expression revealed that *FLH* appears to act in parallel to *FLC*. Surprisingly, the early flowering allele of *FLH* requires the floral integrator *FD*, but not *FT*, to accelerate flowering. This suggests a model in which some alleles of *FLH* are able to affect the FD-dependent activity of the floral activator complex.

POS-TUE-049

REGULATION OF SHOOT APICAL MERISTEM AND COTYLEDON FORMATION BY SEUSS AND SEUSS-LIKE 2

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SEUSS (SEU) and SEUSS-LIKE 2 (SLK2) are components of a transcriptional regulatory complex that promotes shoot apical meristem (SAM) maintenance via a leaf-derived signal. The complex may also be necessary for SAM formation during embryogenesis, as *seu slk2* double mutants fail to form a SAM. Here, we show that *seu slk2* embryos have significantly reduced expression of the *KNOX* genes *SHOOTMERISTEMLESS* and *BREVIPEDICELLUS* (*BP*), whereas other genes associated with SAM development are mostly unaffected. However, restoring *BP* expression in the mutants does not restore SAM formation. One possible explanation is that SEU/SLK2 are required for KNOX activity; consistent with this hypothesis, both proteins physically interact with KNOXs in yeast-based assays. If ongoing BiFC assays confirm these interactions, this will support a model in which KNOXs and SEU/SLK2 function together in a SAM-promoting complex that also perpetuates *KNOX* expression. To identify the tissue/s generating the SEU/SLK2 SAM-promoting signal, we examined the capacity of YFP-SLK2 to complement the meristemless phenotype when expressed in different regions of *seu slk2* embryos. Results indicate that, while SLK2 activity in the abaxial region of cotyledons is sufficient to restore SAM formation, additional signals are required to maintain post-embryonic SAM activity. In addition to loss of SAM formation, *seu slk2* mutants also display other defects such as delayed cotyledon outgrowth. Reporter gene analysis shows that this phenotype is associated with changes in auxin transport and response. SEU has previously been found to interact with ETTIN, a member of the AUXIN RESPONSE FACTOR (ARF) family, and we show that SEU and SLKs also interact with several other ARFs, suggesting that SEU/SLKs regulate auxin signaling through modulation of ARF activity.

POS-WED-050

BRIDGING SIGNALS FROM RECEPTORS TO MAPK KINASES IN STOMATAL DEVELOPMENT

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Stomata are pores on the leaf epidermis that regulate gas exchange between the plant and atmosphere. Proper spatial distribution, as well as the number of stomata in the epidermis, is controlled by asymmetric cell divisions integrated with cell-cell signaling pathways. It was known that the receptor-like protein TOO MANY MOUTH (TMM) and receptor-like kinases in the ERECTA family (ERf) are genetically upstream of the YODA MAPKK kinase in regulation of stomatal development. How these receptors transduce the signal to YODA, however, remains unknown. A forward genetic screen for in a sensitized (receptor-deficient) background lead us to identify VAP-LIKE SUPPRESSOR OF TMM 1 (VST1), a novel component in this system, acting genetically between the receptors and YODA. Although *vst1* mutants have a phenotype only in the original sensitized background, plants lacking VST1 and its two closest homologues, (*vst1;vst2;vst3* triple mutants) exhibited increased amount of stomata on the leaf epidermis. Overexpression of VST1 in *tmm* (and other upstream component) mutants but not *yoda* (or other downstream mutants) restored normal numbers and patterns of stomata to the leaf epidermis. The localization of YFP- Breaking of Asymmetry in the Stomatal Lineage (BASL) translational fusions in triple mutants remained polarized during stomatagenesis, suggesting that the VST family does not function in determining cell polarity. These results suggest that VST family acts as a bridge transducing the signal from receptors to downstream kinase cascade. Mechanisms for how this bridging occurs are under current investigation.

POS-TUE-051

STEROLS ARE REQUIRED FOR THE CELL FATE DETERMINATION OF THE STOMATAL LINEAGE IN ARABIDOPSIS

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Asymmetric cell division is an important strategy to regulate cell proliferation and fate determination during stomatal development in plants. Although a series of genes has been documented to control asymmetric division and cell differentiation in stomatal development, regulators involved in the process between asymmetric division and cell differentiation remain poorly understood. We found that a novel weak allele *fk-J3158* corresponding to the FACKEL (FK) sterol C-14 reductase of Arabidopsis showed clustered small cells and stomata in leaf epidermis. Sterol profile analysis revealed that the mutation in *fk-J3158* blocked downstream sterol production. Further phenotypic investigation indicated that *cyclopropylsterol isomerase1* (*cpi1*), *sterol 14α-demethylase* (*cyp51A2*), and *hydra 1* (*hyd1*) mutants corresponding to enzymes in the same upstream branch of the sterol biosynthetic pathway also displayed defective stomatal development similar to *fk*. Fenpropimorph, an inhibitor of the FK sterol C-14 reductase in Arabidopsis, could mimic these abnormal small cell and stomata phenotypes in wild-type leaves. Genetic experiments demonstrated that sterol biosynthesis is required for correct stomatal patterning through a potential new pathway. The observations and analyses of time-lapse cell division patterns, stomatal precursor cell division markers (*TMM*, *MUTE*, *BASL*), and DNA ploidy suggested that sterols are required for cell fate determination of stomatal lineage cells to properly restrict cell proliferation. This process occurs after physical asymmetric division of the stomatal precursor cells.

POS-WED-052

SH3 DOMAIN-CONTAINING PROTEIN (SH3P) FAMILY MOLECULES ARE INVOLVED IN THE ROOT GRAVITROPIC RESPONSE IN *ARABIDOPSIS*

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AtSH3Ps (SH3-containing proteins) was identified as the molecules that are involved in the trafficking of clathrin-coated vesicles in *Arabidopsis thaliana*. These proteins contain a predicted coiled-coil domain and the Src homology 3 (SH3) domain. In this study, we investigated the function and subcellular localization of SH3Ps using T-DNA insertion or RNAi knockdown mutants and transgenic plants expressing GFP-fusion proteins. We demonstrated that SH3P1-GFP was localized to various organelles including the plasma membrane, transport vesicles as well as the cell plate of dividing cells. In particular, SH3P1-GFP showed polar localization similar to the localization of PIN2 in epidermal cells, and an acitin polymerization inhibitor, latrunculinB, a PI3P kinase inhibitor, wortmannin affected the polarized localization SH3P1. This result indicates that the polar localization of SH3P1-GFP depended on actin cytoskeleton and the endocytotic pathway. We also showed that SH3P3-GFP was predominantly expressed columella cells, and localized to the specific domain of the plasma membrane. The localization of SH3P3-GFP changed after gravity stumulus. The single knockout or knockdown mutants of SH3Ps showed a severe defect in root gravitropic responce. Taken together these results, we concluded that SH3Ps play important role in root gravitropism.

POS-TUE-053

CHANGES IN DISTRIBUTION OF CELL WALL POLYSACCHARIDES IN FLORAL AND FRUIT ABSCISSION ZONES DURING FRUIT DEVELOPMENT IN TOMATO

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After fruit development has been triggered by pollination, the abscission zone (AZ) in the pedicel strengthens its adhesion to keep the fruit attached. Unpollinated flowers are shed at their respective AZs, whereas an enlargement of the same tissue is observed in pollinated flowers. After the fruit has developed and is fully ripened, shedding occurs easily at the AZ, indicating an acceleration of abscission. Cell wall degradation and synthesis may play important roles in these processes. We have visualized changes in polysaccharide distribution in the AZs of pollinated versus unpollinated flowers and in the ripened fruits using immunohistochemistry. During floral abscission, a large increase was observed in LM15 labeling of xyloglucan and LM5 and LM6 labeling of galactan and arabinan, specifically at the AZ in the abscising pedicel. The results suggest that xyloglucan, pectic galactan and arabinan play key roles in the floral abscission. During fruit abscission, no AZ-specific cell wall polysaccharide deposition was observed; however, lignin staining was seen in the AZ of over-ripe fruit pedicels, suggesting lignification of the AZ prior to fruit abscission.

POS-WED-054

PROMOTION OF CHLOROPLAST PROLIFERATION UPON ENHANCED POST-MITOTIC CELL EXPANSION IN LEAVES

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Leaves are determinate organs: hence, precise control of cell proliferation and post-mitotic cell expansion is essential for their growth. A defect in cell proliferation often triggers enhanced post-mitotic cell expansion in leaves. This phenomenon is referred to as compensation. Several lines of evidence from studies on compensation have shown that cell proliferation and post-mitotic cell expansion are coordinately regulated during leaf development. Therefore, compensation has attracted much attention to understand the mechanisms for leaf growth. However, our understanding of compensation at the subcellular level remains limited because studies of compensation have focused mainly on cellular-level phenotypes. To gain insight into the subcellular aspect of compensation, we investigated the well-known relationship between cell size and chloroplast number per cell in compensation-exhibiting lines. We first established a convenient method for observation of chloroplasts *in situ*. Using this method, we analyzed *Arabidopsis thaliana* mutants *fugu5* and *angustifolia3* (*an3*), and a transgenic line *KIP-RELATED PROTEIN2* overexpressor (*KRP2* OE), which are known to exhibit typical features of compensation. We showed that chloroplast number per cell increased in the subepidermal palisade tissue of these lines. We found that promotion of chloroplast proliferation depends on the enhanced post-mitotic cell expansion in *fugu5* and *an3*. We analyzed tetraploidized wild type, *an3* and *KRP2* OE, and found that cell size itself, but not nuclear ploidy, is a key parameter that determines the activity of chloroplast proliferation. Furthermore, we revealed that the expression profile of *PLASTID DIVISION* genes, key regulators of chloroplast proliferation, in compensation-exhibiting lines is similar to that in the wild type, arguing as-yet-unknown mechanism which is responsible for modulation of chloroplast proliferation.

POS-TUE-055

VARIATION IN THE LEVEL OF HETEROSIS AMONG F_1 HYBRID INDIVIDUALS IN *ARABIDOPSIS THALIANA*

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Heterosis (or hybrid vigor) is a phenomenon in which hybrid progeny exhibit superior performance to their parents. Heterosis has been used in many crops and vegetables to make high-yielding cultivars, but the molecular mechanism(s) of heterosis is still to be elucidated and there is no agreed model. Some *Arabidopsis thaliana* accessions such as Columbia (Col) and C24 show heterosis in biomass of the F_1. Among the F_1 hybrids between Col and C24, there is variation in shoot size, and the biggest rosette diameter is 2.7 times larger than smallest at the 10 days after sowing. The big plants had an approximately 80% reduction in the number of palisade mesophyll cells per unit area relative to the medium sized plants, indicating that the larger leaf area of the big plants is due to increased cell size. To identify differentially expressed genes between the different sized plants, we compared the transcriptome of big and medium sized plants at 10 days after sowing, using the Affymetrix, Arabidopsis ATH1 Genome Array. 441 probe-sets showed 1.5-fold difference in expression. Genes involved in “stress response” or “response to stimulus” tended to show differential expression. Adaptation to altered environmental conditions is important for achieving a high level of heterosis, and the differentially expressed genes that we found may contribute to stabilize the high level of heterosis.

POS-WED-056

FUNCTION OF KIP-RELATED PROTEINS OF ARABIDOPSIS DURING PLANT DEVELOPMENT

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The cell cycle plays an important role in the development and environmental adaptation of multicellular organisms; specifically, it allows them to optimally adjust their architecture in response to environmental changes. Kip-related proteins (KRPs) are important regulators of cell cycle and endoreduplication in plant development. The Arabidopsis genome possesses seven *KRP* genes with low sequence similarity and distinct expression patterns; however, how these genes function in cell cycle regulation is largely unknown. Here, we focused on the characterization of KRP functions and their expression patterns during plant development. KRP3 protein was localized to the SAM, including the ground meristem and vascular tissues in the ground part of the SAM and cotyledons, whereas KRP6 protein was expressed weakly in the proximal side of leaf primordia. In addition, KRP proteins were stabilized when treated with MG132, an inhibitor of the 26S proteasome, indicating the protein may be regulated by 26S proteasome-mediated protein degradation. *KRP* overexpressing transgenic plants had a higher DNA ploidy level in the SAM and leaves. Taken together, we will discuss the roles of KRP proteins in cell cycle and endoreduplication during developmental changes.

POS-TUE-057

LARGE SCALE GENE EXPRESSION ANALYSIS IN ABC MODEL HOMEOTIC MUTANTS

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The most important model describing flower development is a widely known ABC model which explain how interactions of transcription regulators result in specification of floral organs. Many efforts were applied for deeper understanding of the ABC model on the past twenty years. Nevertheless for now little is known about genes downstream to regulators of ABC model. We performed large-scale analysis of gene expression in inflorescences of mutants *ag-1*, *pi-1*, *ap3-6*, *ap2-7*, *ap2-1*, *ap1-1* by transcriptome sequencing (RNA-seq). Sequencing was performed using Illumina HiSeq2000 in two biological replicates. We obtained from 20 to 35 millions of reads for each sample and more than 97% of reads were successfully mapped to exons. R package DESeq was used for identifying differential expressed (DE) genes. The number of DE genes was from 1596 for *ap1-1* to 4499 for *ap3-6*. To avoid false positive results caused by difference in ratio of different organs and tissues between objects being compared (so called pattern effect), DE genes were processed by the filter that we developed. Also this filter allows discovering genes specific for each type of floral organs. After filtration of genes prone to pattern effect genes regulated by ABC genes were identified. The number of such genes varies from 597 for *ap1-1* to 1310 for *ap3-6*. With analysis of intersections between different genes' sets genes controlled by single ABC gene or by group of ABC genes were found. Analysis of Gene Ontology enrichment of DE expressed genes reveals association between control of meristem maintenance and stress response genes. Also control of meristem activity as in case of *AG* and *AP2* is linked with genes involved in membrane formation. This result raises hope that processes regulated by genes of ABC model can be deeply understood in light of new sequencing technologies.

POS-WED-058

REGULATION OF FLOWERING IN ARABIDOPSIS BY *VOZ* TRANSCRIPTION FACTORS

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The decision to flowering is an important developmental parameter in plant life. The onset of flowering, therefore, is strictly regulated by a complex network of pathways that co-ordinate and respond to various endogenous and environmental stimuli to finally converge on a few floral integrators leading to flowering. Here, we aim to dissect the role of two homologous genes, *VASCULAR PLANT ONE ZINC FINGER PROTEIN1 & 2 (VOZ1 & 2)* in Arabidopsis. The *voz1voz2* double mutant exhibits late-flowering while the over-expression of either of the genes confers an early-flowering phenotype, demonstrating that *VOZ1 & 2* promote flowering. Expression analysis of known flowering regulators in the *voz1voz2* double mutant revealed that *VOZ1 & 2* promote flowering by negatively regulating the expression of *FLOWERING LOCUS C* gene. Both *VOZ1 & 2* exhibit a diurnal rythmicity in their expression at the mRNA level, suggesting that *VOZs* are associated with the *Arabidopsis* circadian clock. Moreover, total starch content, which is under circadian regulation, is reduced in the double mutant, giving credence to the link between circadian clock and *VOZ*. Expression analysis indeed shows increased *VOZ1* and *VOZ2* transcripts in *cca1-11;lhy-21*, a double mutant where the clock function is abolished. Bioinformatic analysis revealed the presence of a single CCA1-binding site (CBS) in the upstream regions of *VOZ1* and *VOZ2* and recombinant CCA1 protein is capable of binding to these *cis*-elements in EMSA experiments, suggesting that *VOZ* expression is under direct circadian control. Our study, therefore, reveals a new link between the circadian clock and *FLC*-mediated regulation of flowering time.

POS-TUE-059

LIVE-CELL ANALYSIS OF EMBRYOGENESIS IN *ARABIDOPSIS THALIANA*

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Multicellular animals and plants develop from the single-celled zygote to form the mature embryo. During embryogenesis, the zygote follows a pattern of cell division to form the body plan of the embryo. However, the confined nature of plant embryogenesis processes, which occur in the female reproductive organs and several cell layers of the pistil, limits our ability to observe these events *in vivo*. Here, we observed the cell division during embryo development in *Arabidopsis thaliana* by live-cell imaging *in vitro*. First, we succeeded in time-lapse recordings of cell division in heart-stage and torpedo-stage embryos using isolated embryos expressing fluorescent protein labeled histone H2B. However, cell division occurred only during late embryogenesis in this *in vitro* system. Next, we used the isolated ovules to observe the cell division in early embryogenesis. So far, we succeeded in time-lapse recordings of cell division in isolated embryos for more than 2 days using spinning disk confocal microscopy. For further long-term live-cell imaging of embryogenesis, we need to use two-photon excitation microscopy. As the embryo and endosperm develop, the ovule expands and the distance of the embryo from the glass surface increases to approximately 100-200 µm. Thus, a system in which there is less cell damage and that allows deep imaging is necessary for long-term live-cell imaging of embryogenesis. Moreover we are currently performing optical manipulation for spatio-temporal gene expression and protein inactivation in this *in vitro* system.

POS-WED-060

RAPTOR, A HIGHLY CONSERVED ELEMENT OF THE EUKARYOTIC TOR KINASE GROWTH PROMOTING COMPLEX, IS NOT ESSENTIAL FOR PLANT SURVIVAL

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Target of Rapamycin (TOR) is a highly conserved protein kinase found in all eukaryotes that regulates growth in response to nutrient availability and other extrinsic cues. Though most intensively studied in yeast and metazoans, TOR is also essential for growth in plants. Given the independent evolution of multicellularity in plants, we are interested in the degree to which their growth and survival depends on TOR signalling. Our analysis has initially focused on RAPTOR (Regulatory Associated Protein of TOR), a component of the TOR kinase Complex 1 (TORC1), which promotes growth by activating translation machinery related targets. Disruption of RAPTOR in animals and the RAPTOR homologue in yeast is lethal. By contrast, in our analyses of RAPTOR in Arabidopsis, we can isolate viable lines in which the two genes encoding RAPTOR proteins have both been disrupted. We present an analysis of these and other lines, detailing defective growth phenotypes that result from decreased RAPTOR activity. We also present studies detailing hypersensitivity of RAPTOR deficient lines to specific kinase inhibitors, and discuss how these may contribute to understanding of the nature of growth enabling pathways in plants.

POS-TUE-061

THE NEW FINDING OF ARABIDOPSIS *GRF-INTERACTING FACTOR* GENE FAMILY IN DEVELOPMENT OF CARPEL AND OVULE

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A inflorescence and floral architecture are developed in reproductive process in *Arabidopsis thaliana*. Previously, the *GRF-INTERACTING FACTOR* (*GIF*) transcription coactivator gene, a member of small gene family comprising three genes, were characterized as a positive regulator of cell proliferation in lateral organs, such as leaves and flowers, of *Arabidopsis thaliana*. The *gif* triple mutant flower had reduced number of petals and stamens, i.e., on average, about 2.1 and 4.4, respectively. Most prominently, *gif* triple mutant developed defective floral organs. Mutant carpels failed to fuse together, resulting in a split gynoecium. It turned out that the gynoecial defect was caused by a precocious differentiation of replum tissues, which ultimately results from aberrant activities of the meristematic medial ridges. Ovule development of *gif* triple mutant was also severely affected: emerging integuments lost cell proliferation activities and the adaxial-abaxial polarity and eventually failed to surround the nucellus. Additionally, the mutant did not develop functional embryo sac and pollen. Also, *gif12 ant-1* triple mutant developed split carpels like *gif* triple mutant, parental lines did not. GUS expression of *GIF* gene family is correlated with abnormal phenotypes. In conclusion, the *GIF* gene family is a novel and essential component for development of reproductive organs, allowing normal life cycle of Arabidopsis.

POS-WED-062

MADS-BOX PROTEIN COMPLEXES ACT AS THERMAL MEDIATORS IN DIFFERENT AMBIENT TEMPERATURE REGIMES

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Flowering time is one typical example of developmental plasticity, however, little is known about the molecular mechanism underlying ambient temperature-responsive flowering. Here, we demonstrated that a direct mechanism by which decreasing or increasing temperature causes the MADS-box proteins to repress expression of ambient temperature integrators. We found that Nd ecotypes with naturally deleted *FLOWERING LOCUS M* (*FLM*) genomic locus showed similar early flowering phenotype at 23°C and 16°C. Furthermore, genetic studies revealed that a lesion in *FLM* resulted in ambient temperature-insensitive flowering phenotype. Interestingly, *short vegetative phase* (*svp*) mutants did respond to broad ranges of ambient temperature, whereas *flm* and *flowering locus c* (*flc*) mutants did respond to narrow ranges of ambient temperature. Expression analysis and genetic interaction studies suggested that *FLM* and *SVP* act upstream of *FLOWERING LOCUS T* (*FT*), *TWIN SISTER OF FT* (*TSF*), and *SUPPRESSOR OF OVEREXPRESSION OF CONSTANS1* (*SOC1*), the ambient temperature integrartors. Chromatin immunoprecipitation (ChIP) analysis showed that FLM and SVP proteins directly bound to the genomic regions of *FT*, *TSF*, and *SOC1*. Furthermore, protein-protein interaction studies showed FLM and FLC proteins physically interacts with SVP protein *in vitro* and *in vivo*. Although the difference in *SVP* RNA expression levels was not very apparent at different temperatures, the stability of SVP protein was significantly changed. Furthermore, temperature affected the formation of the MADS-box protein complex containing SVP, FLM, and FLC protein, and eventually the binding of these MADS-box protein complexes to genomic regions of FT, TSF, and SOC1. Taken together, we propose that the MADS-box protein complexes act as important thermal mediators within the ambient temperature pathway.

POS-TUE-063

SERKS CONTROL EMBRYO DEVELOPMENT VIA MAPK SIGNALING PATHWAY IN ARABIDOPSIS THALIANA

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Receptor-like protein kinases (RLKs) are single-pass transmembrane protein kinases critically involved in cell-to-cell and cell-to-environment communications. There are more than 400 typical RLKs in *Arabidopsis*. Based on their extracellular domain structures, RLKs were classified into 13 subfamilies. Among them, leucine-rich repeat (LRR) RLKs belong to the largest subfamily, possessing at least 223 members; somatic embryogenesis receptor kinases (SERKs) are a small group of LRR-RLKs, consisting of only 5 members. The first *SERK* was identified as a marker gene to monitor cell identity change from somatic to embryonic cells in carrot suspension cultures. Studies from several labs indicated that *Arabidopsis* SERKs play important roles in regulating distinct signaling pathways such as brassinosteroid (BR) signaling, cell death control, and various innate immunity responses. Recent loss-of-function genetic studies from our lab indicated that *serk1 serk2 serk3 serk4* quadruple mutant showed an embryo lethality phenotype, whereas triple mutant *serk1 serk2 serk3* displayed delayed development and aberrant embryo phenotype. These observations provide strong evidence that SERKs are in deed essential to *Arabidopsis* embryo development. The embryonic defects become evident at the stage from globular to heart-shaped embryos. Further analyses showed that the expression patterns of a quiescent center marker gene, *WOX5*, are altered in the triple mutant embryos compared to those in wild type embryos, with dramatically enhanced expression in the provascular cells. Our data also showed that SERKs-mediated signals are transduced via an YDA-MKK5-MPK6 cascade to control *Arabidopsis* embryo development. These results suggest that SERKs control embryo development via a MAPK cascade by finely tuning and restricting the *WOX5* expression in quiescent center cells.

POS-WED-064

REGULATION OF THE *MLP* GENE FAMILY MEMBERS, BY TWO LAYERS OF TISSUE SPECIFIC DEGRADATION, PLAY A DEVELOPMENTAL ROLE IN *ARABIDOPSIS*

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The microRNA394 (miR394) family is highly conserved in plants and regulates the F-box protein gene *LCR* (*Leaf Curling Responsiveness*). F-box proteins act as post-translational modulators and generally target specific proteins for ubiquitination and degradation; however the identification of the target protein(s) of the F-box LCR has been challenging. Analysis of reporter gene expression reveals overlapping expression domains of *MIR394* and *LCR*. Plants transformed with a miR394-resistant target, leading to overexpression of the *LCR* (OE), display downward leaf curvature, and eventually shoot apical meristem termination. Plants in which *LCR* has been disrupted by T-DNA insertion (KO) have the opposite effect, displaying only mild upward curvature. These phenotypes correlate with the abundance of *LCR* transcript levels. To identify the proteins regulated by the LCR, proteomic analysis was carried out by stable isotopic labelling and tandem mass spectrometry. This approach identified proteins that are differentially expressed in the OE and KO lines when compared to wild-type (WT). A total of 4291 protein groups were quantified, however only seven proteins were significantly altered in abundance between the OE and KO lines. Two of these, MLP and SOUL protein, gave the expected inverse reciprocal expression for OE/WT and KO/WT ratios. To further validate these candidates, epitope-tagged versions of the proteins were transiently co-expressed in *Nicotiana benthamiana* leaves along with the LCR protein. Western blotting results identified MLP as a putative bona fide target of the F-box LCR. This work shows that the biological role of miR394 is to ensure de-repressed expression of MLP in appropriate tissues and that this is required for normal *Arabidopsis* development.

POS-TUE-065

EPIGENETIC CONTROL OF CALLUS REGENERATION IN *ARABIDOPSIS*

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Plant de novo organogenesis could be achieved via the callus regeneration pathway. Although several lines of evidence support the notion that callus is the root-tip-like tissue, very little is known about the underlying mechanisms that control the cell fate transition from different kinds of explants to callus. We previously reported that the leaf-to-callus transition requires at least three steps of processes: 1) activation of auxin-related genes; 2) silencing of leaf genes; and 3) activation of root genes. By analyzing callus formation of Arabidopsis mutants corresponding to different epigenetic pathways, we found that leaf blades of the Polycomb group (PcG) and the Imitation Switch (ISWI) type ATP-dependent chromatin remodeling factor mutants were both defective in callus formation. More detailed studies demonstrated that these two epigenetic pathways act independently at the different steps of callus induction. While PcG silences the leaf characters, ISWI promotes the root meristem cell fate. Our data indicate that epigenetic control of cell fate transition is critical for callus regeneration.

POS-WED-066

THE TRANSCRIPTIONAL NETWORK ANALYSIS OF ADAXIAL-ABAXIAL POLARITY IN ARABIDOPSIS LEAF DEVELOPMENT

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The evolutionarily conserved adaxial-abaxial regulatory network controls some of the most basic aspects of plant growth and function, including formation of meristems in the shoot and root, branching and cell type development. The establishment of adaxial-abaxial polarity is determined by differential gene expression regulated by master regulators that include the plant-specific *HOMEODOMAIN-LEUCINE ZIPPER class III* (*HD-ZIPIII*, promotes adaxial fates) and the oppositely acting *KANADI* (*KAN*, promotes abaxial fates) gene families. To identify targets of REV (an HD-Zip class III transcription factor) and KAN1, microarray analysis was used to determine changes in gene expression at various times after dexamethasone induction of GR-REV and KAN1-GR transgenic plants. Our analysis shows substantial overlap between the ad/abaxial network and hormone signaling pathways with ABA and auxin being especially prominent leading to models for ABA and auxin action in the development of ad/abaxial differences in growth. Our analysis has also identified many transcription factors - where these have known function, they indicate the developmental subprograms that are activated (or repressed) differentially in ad and abaxial domains. In several cases, our analysis has uncovered transcription factors of unknown function - we are currently exploring the function of these in leaf, shoot apical meristem and embryo development.

POS-TUE-067

FUNCTIONAL CHARACTERIZATION OF POLLEN-SPECIFIC KINASES THROUGH THE GENERATION OF KNOCK-DOWN TRANSGENIC PLANTS EXPRESSING HAIRPIN-RNAS (HPRNAS) AND AMIRNAS

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Pollen grains are the male gametophyte of plants and thus are essential for plant reproduction and productivity. However, despite their biological and agronomical importance, little is known about the molecular mechanisms that regulate its development and function. In Arabidopsis, three cells compose mature pollen grains: a large vegetative cell and two small sperm cells engulfed in the cytoplasm of the vegetative cell. During fertilization, the vegetative cell must germinate and produce a pollen tube, a growing tip structure that directionally transports the sperm cells to the ovule to produce the double fertilization event. Currently, little is known about signal transduction pathways and molecular components involved in these processes. Using microarray data we have previously identified 4 genes encoding kinases proteins (*PSK1* to *4*, for *POLLEN SPECIFIC KINASE*) that are expressed exclusively during the last stages of pollen development, germination and tube elongation. To analyze the physiological relevance of these genes, we have generated transgenic plants expressing specific ihpRNAs and amiRNAs for these genes under the control of a pollen-specific promoter (LAT52) and we have analyzed pollen development and tube elongation in insertional mutants and transgenic plants expressing ihpRNAs and amiRNAs. Also, we have determined the sub-cellular localization of each kinase protein using 35S:*PSK*:GFP and LAT52:*PSK*:GFP constructions in agroinfiltration experiments. Taken these together, we present 4 pollen specific kinases required for pollen development and tube elongation in Arabidopsis thaliana. Funded by Fondecyt 1120766 and UNAB DI-74-12/R.

POS-WED-068

A ROS RESPONSIBLE TRANSCRIPTION FACTOR REGULATES ROOT GROWTH VIA ABA SIGNALING

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ROS homeostasis in the root tip is one of the keys for regulating the balance between the cell proliferation and the cell differentiation. However little is known about the transcriptional network regulated by the ROS. To understand the gene regulation of the ROS signaling, we performed time course microarray analysis by using Arabidopsis root treated by the H_2O_2 for 1, 3 and 6 hours. We found about 200 genes which showed clear response to H_2O_2. Among them, we focused on one transcription factor which was named ROS First Response TF 1 (RFRT1). *RFRT1* expression was upregulated by the H_2O_2 treatment within one hour. In the *RFRT1* transcriptional fusion of GFP, the fluorescence increased and expanded the expression domain in the root tip by the H_2O_2 treatment in the short period. We investigated the root elongation rate of T-DNA insertion line of *RFRT1* upon H_2O_2 treatment. *rfrt1* mutant showed slightly higher sensitivity to the H_2O_2 than the wild type. To know the hormonal effects on the *rfrt1* root growth, we treated *rfrt1* root with IAA, trans-zeatin and ABA. IAA and trans-zeatin inhibited *rfrt1* root growth as same level as wild type. However, *rfrt1* root showed clear insensitivity to ABA treatment, that treatment apparently inhibited wild type root growth. The transcriptional fusion was upregulated by the ABA treatment, but the increment of the promoter activity was lower than the H_2O_2 treatment. According to these results, we hypothesized that RFRT1 may have a function as a rheostat between ABA and ROS signals that regulates root growth via the balance between the cell proliferation and cell differentiation.

POS-TUE-069

PHOTOPERIODIC COMPENSATION: A MECHANISM UNDERLYING REGULATION OF CHLOROPHYLL AMOUNT BY CIRCADIAN CLOCK IN *ARABIDOPSIS THALIANA*

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Chlorophyll *a* and *b* are essential pigments for photosynthesis of higher plants. Two related myb proteins, LATE ELONGATED HYPOCOTYL (LHY) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1), play key roles in the clock-controlled biological processes in *Arabidopsis thaliana*. As a novel example of the phenomenon of biological compensation, here we show that total amount and *a/b* ratio of chlorophyll are compensated by photoperiod in *Arabidopsis thaliana*. Double loss-of-function of LHY and CCA1 genes (*lhy;cca1*) significantly alters photoperiodic compensation of both the total amount and *a/b* ratio of chlorophyll. The defect of photoperiodic compensation in *lhy;cca1* is largely suppressed by a mutation of a floral repressor gene, SHORT VEGETATIVE PHASE (SVP). Our results indicate that LHY, CCA1 and SVP play key roles in the control of photoperiodic compensation of chlorophyll as well as flowering time and organ elongation.

POS-WED-070

CHARACTERISATION OF MOLECULAR FACTORS DETERMINING ASYMMETRY IN FLOWERS OF *ARABIDOPSIS THALIANA*

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The formation of correct flower structure is important for the reproductive success of plants. Asymmetrical flowers – flowers which differ in shape or size from the corresponding wild type – do not only lead to a reduced, but sometimes also to higher reproductivity. To identify and characterise factors, which cause flower asymmetry, two single-copy *Ds* transposon insertion lines of *Arabidopsis thaliana* with abnormal flowers were identified. The flowers of these mutant lines differ phenotypically significant from the corresponding wild type. The first mutant shows a completely altered inflorescence and an abnormal composition and incomplete formation of floral organs, whereas it's vegetative growth is normal. The flowers of this mutant do not only lack the white coloured petals but also have stamen which are partially converted into small secondary flowers and shows insufficiently fused carpels leading to a reduces seed production and disturbed germination. The flower organs of the second mutant are normal, but their overall as well as the seed size is larger than those of the corresponding wild type. The vegetative growth of the mutant again displayed no difference to the wild type. Our data of the molecular characterisation of these mutant lines will be presented and discussed. Our results may lead to new insights into the formation of asymmetric flowers.

POS-TUE-071

THE REGULATORY ROLE OF ATMYB5 IN SEED COAT AND TRICHOME DEVELOPMENT

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Arabidopsis trichomes and seed coat are useful models to study cell wall structure and development. These structures can be used to identify key regulators of two major plant cell wall constituents, cellulose and pectin. One of the major links between trichome and seed coat structure is the carbohydrate pectin. A regulator of pectin synthesis in seed coat development is the MYB transcription factor AtMYB5. In the seed coat pectin is found in the form of mucilage, which is located between the primary and secondary cell walls and released upon imbibition. Seeds of an atmyb5 T-DNA knockout mutant produce significantly less mucilage than wild type. Redundancy between AtMYB5 and a similar R2R3 MYB transcription factor, AtMYB23, was uncovered. AtMYB23 regulates trichome morphology, more specifically branching and cell expansion. atmyb23 knockout mutants exhibit trichomes which contain fewer branches (1-2) than their wild type counterparts (3-4). An *atmyb5/atmyb23* double knockout mutant exhibited a more severe reduction in cell expansion and branching. Key transcriptional regulators under the control of AtMYB5 and AtMYB23 were identified in trichome development. Furthermore, several enzymes which belong to the pectin methylesterase and Galacturonosyl transferase families were discovered. T-DNA mutants of these genes were obtained with one mutant phenotype revealing an important role in trichome development. Redundancy between AtMYB5 and AtMYB23 in trichome development highlights a potential overlap in the regulatory pathways which control pectin and cell wall synthesis in various parts of the plant. From a theory based on the synthesis of two key cell wall components, pectin and cellulose, an overlapping developmental network has been uncovered.

POS-WED-072

THE TALE OF GALT14 AND ITS POSSIBLE ROLE IN AGP GLYCAN BIOSYNTHESIS IN *ARABIDOPSIS THALIANA*

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Arabinogalactan-proteins (AGPs), members of the hydroxyproline-rich superfamily, are proteoglycans that are found at the cell surface (the plant cell wall and plasma membrane) and in secretions. AGPs have been proposed to have a variety of functions *in planta*. In *Arabidopsis thaliana*, these proteins are likely to be important in the initiation of female gametogenesis (Acosta-Garcia and Vielle-Calzada, 2004), signal transduction pathways regulating plant growth and development (Zhang et al., 2011), modulation of phytohormone activity responsible for root growth and seed germination (van Hengel and Roberts, 2003), pollen tube germination (Pereira et al., 2006), and cell division and cell expansion (Yang et al., 2011). In general, AGPs are characterized by the presence of arabinose and galactose as the predominant sugars in their carbohydrate moiety. How the carbohydrate decoration of AGPs is synthesised is not well understood, and only a few glycosyltransferases (GTs) have been associated with its synthesis. One GT family, GT31, has been predicted to form β-(1,3)-Gal linkages (Qu et al., 2008). Our research aim is to explore the functions of GT31 members GalT12 and GalT14 in the formation of β-(1,3)-Gal linkages and determine whether they have roles in AGP biosynthesis. Through genotyping and phenotypic analysis of a *galt14* T-DNA insertion mutant, we have found this line has a pollen defect. The characterization of the galt14 mutant will be described and its potential role in pollen grain wall formation discussed.

POS-TUE-073

APPROACHES TO INTEGRATE NITROGEN SIGNALS INTO THE FLOWERING NETWORK

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Nitrogen (N) is an essential nutrient for plants, typically representing 2-5% of the plant dry weight. The N supply often limits plant growth and development. Next to developmental processes like germination, shoot-root allocation, lateral root growth, senescence, the concentration of nitrate as the major source of N in the soil has been known for almost a century to modify the timing of flowering in plants. Marin and coworkers suggested in a recent publication that N influences flowering via a novel signaling pathway that includes nitrate or a substance that is metabolized from nitrate (Marin et al., 2011). At which point the N signal, which is thought to act in a separate pathway, interacts with the known floral induction pathways is not known to date. In the work presented here, we aim at getting a better insight into how the N signal is to be integrated into the existing flowering network.

POS-WED-074

DEVELOPMENT OF VEGETATIVE AXILLARY BRANCHES AFTER VERNALIZATION IN *ARABIS ALPINA*, PERENNIAL PLANT

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Annual plants are flowering only once then complete their life cycle. In contrast, perennial plants show flowering repeatedly and live for several years. The *Arabis alpina* is one of perennial species, which is closely related to *Arabidopsis thaliana*. *A.alpina* Pajares accession requires long-term of vernalization treatment for phase transition from vegetative growth to polycarpic reproductive growth. Another special feature of *A.alpina* is actively branching phenotype. Most of axil buds at individual leaf axils can develop into axillary branches. Axillary meristem (AM)s develop into axillary buds and later generate axillary branches. Shoot branching is activated by molecular regulators and controlled by systematic hormonal signals. Recently, several genes were revealed as molecular regulators for AM formation in *A.thaliana*. The *REGULATORS OF AXILLARY MERISTEMS* (*RAX*) genes were R2R3Myb family members, which have a redundant role for promoting axillary buds. The abscisic acid (ABA) is a phytohormone that plays important roles in plant development, including seed maturation and dormancy. In addition, ABA causes physiological responses to environmental stresses, for examples, stomatal closure induced by drought or osmotic stress, and inhibition of growth during abiotic or biotic stress conditions. Our research focused on regulating factors activating vegetative AM in polycarpic perennials after vernalization. We performed transcriptome-analysis using AM triggered in the primary stems of *A.alpina* after vernalization. Accoring to the result, about 1,000 genes were up-regulated after vernalization. Among them, many ABA-related genes and cold induced stress responsive genes were detected. The expression of *RAX* homologous genes was also increased in vernalized *A.alpina* stems. Besides, *AaRAX2* was transcriptionally activated by exogenous ABA treatment. These results show that cold-induced ABA signaling might stimulate formation of vegetative branches through *AaRAX2* activation during vernalization.

POS-TUE-075

USE OF A NOVEL FLUORESCENT BASED SYSTEM TO INVESTIGATE PROTEIN-PROTEIN INTERACTIONS OF PETAL LOSS

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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 apparently to suppress growth in these regions. Overgrowth in *ptl* mutants disrupts nearby petal initiation. PTL is a member of the plant specific tri-helix family of transcription factors. The PTL protein contains two putative trihelical DNA binding domains. Located between these is a central conserved domain predicted to form a coiled coil, likely involved in protein-protein interactions. We have previously shown that, as with other members of this family, PTL can homodimerize. Protein-protein interactions are important for the function of many proteins. Studying these interactions however can be problematic. Bimolecular Fluorescence Complementation (BiFC) is a widely used tool to study such interactions which has many advantages over other methods such yeast two hybrid tests as it can be performed rapidly and *in planta*. However BiFC has its limitations also. These include a relatively high frequency of both false positives and false negatives, often associated with difficulty in identifying appropriate useful controls. To overcome these limitations we have developed a novel *in planta* fluorescent based system with a high signal to noise ratio. Potential partners are tagged with different intra-cellular localization sequences and different full-length fluorochromes. Positive interactions are indicated by shifts in cellular localization. Using this system we have mapped regions of the PTL protein involved in homodimerization as well as confirming several interacting heterologous partners.

POS-WED-076

TISSUE SPECIFIC REQUIREMENT OF TOR SIGNALLING IN PLANTS

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Throughout all eukaryotes, the TOR pathway (Target Of Rapamycin) represents an ancient and fundamental mechanism to regulate cellular growth. In this signalling pathway, the serine-threonine kinase TOR represents a central element, which is sensitive to energy levels, nutrients, stress and growth factors. TOR activity promotes an output of coherent growth through phosphorylation dependent regulation of translation, metabolism and cytoskeletal organisation. Through its involvement in cancer, diseases and aging, mammalian TOR has been intensively studied, while its role in plants is much less understood. To date, most work in plants has focused on elements of TORC1 (TOR complex 1), which includes TOR, as well as the accessory proteins RAPTOR and LST8. Initial studies have shown that like animals and fungi, TORC1 is necessary for normal regulation of growth, and acts to up-regulate translational outputs. However, many questions relating to TOR function in plants remain. Our work aims to further clarify the function of individual elements of the TOR pathway in plants, especially with respect to plant specific patterns of multicellular growth. Experiments with tissue specific RNAi directed against TOR suggest significant differences between tissues in their requirement for TOR. To extend this approach further, we have adapted a CRE/lox recombination system to create mosaic plants that contain marked sectors in which TOR signalling elements are deleted. This work should provide a more definitive description of the tissue and stage specific requirements for TOR signalling in plants.

POS-TUE-077

CHARACTERIZATION OF ROOT CELL IDENTITY THROUGH GENOMIC LOCALIZATION OF T-DNA INSERTIONS IN *ARABIDOPSIS THALIANA* GAL4-GFP ENHANCER TRAP LINES

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In recent years, great strides have been made in understanding the root growth and development of *Arabidopsis thaliana* but have been mostly focused on a physiological, proteomic or transcription factor network level. Our approach takes a look at root development through a genomic perspective utilizing the tissue specific expression of the reporter gene, Green Florescence Protein (GFP), in a subset of the mutant *Arabidopsis thaliana* plants known as the GAL4-GFP enhancer trap lines. Given the mostly unknown genomic nature of our selected mutated plants, characterizing the tissue expression organization at the genomic level will expand established models of gene expression spatiotemporal maps and cell fate by gradients. Thermal Asymmetric Interlaced PCR is being used to recover the promoter sites of the T-DNA inserts used to create the tissue specific expression in the GAL4-GFP enhancer trap lines. Following mapping, respective GFP expressing root cell protoplasts will be harvested to examine the retention of the cell identity in relation to the location within the genome. The study looks at how the overall *A. thaliana* genome is being orchestrated during development for the final tissue specific expression of a select number of enhancer trap lines as a model for understanding root development. (This research was funded by the NIH T34 GM 08395-22 grant).

POS-WED-078

RAPID SCREENING FOR PHOTO-PROTECTIVE MECHANISM IN ARABIDOPSIS USING FLUORESCENCE IMAGING

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In the last few decades, the use of chlorophyll fluorescence has becomes a very powerful method for monitoring photosynthetic performance in plant. Here we develop a high-resolution and high throughput analysis pipeline photo-protective phenotyping using chlorophyll fluorescence and TrayScan (PSI Instruments) that can automatically measure 300 plants. One of photo-protective parameters that we are studying is non-photochemical quenching (NPQ). It is the mechanism that plant dissipates excess absorbed light energy as heat which is harmless to the plant. At first, we conducted experiment with 20 Arabidopsis accessions. We found that there are variations of NPQ levels among 20 accessions. Moreover, to identify genetic factors responsible for NPQ variation, we undertook QTL mapping with recombinant intercross lines (RIXs) which were a cross between C*vi* and L*er*. The result showed that there are correlations of makers that relate to NPQ. Thus, preliminary experiments indicate that high throughput fluorescence imaging can be used to study photo-protection and to find new genes and alleles that regulate its induction.

POS-TUE-079

TRANSCRIPTIONAL CONTROL OF ROOT CAP DIFFERENTIATION

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Organ size control boils down to control the balance between cell differentiation and the cellular growth processes, cell proliferation and expansion. It is shown this balancing involves a strict control of hormone gradients that dictate the spatiotemporal position where differentiation starts and the growth processes end. Transcript profiling revealed that the transition to differentiation coincides with vast transcriptional changes. However neither the necessity nor the key transcriptional regulators of this transcriptional reprogramming are known. To get a better understanding of the transcriptional changes at the transition, we set up a screening for the identification of transcription factors essential for proper organ growth regulation. For the identification, we employed a system to over come functional redundancy: a collection of 1400 transcription factors was dominantly repressed by fusing them to the SUPERMAN repression domain. In this collection several candidates were selected based on altered growth characteristics. Now we are investigating the candidates' roles in organ size control. Ectopic expression studies and transcript profiling indicates one of the candidates is able to repress root cap cells to differentiate and keep cells in a proliferation state. We believe this gene brakes differentiation. Genome wide expression analysis together with genetic studies will reveal how this brake mechanistically affects differentiation and identify the genes involved.

POS-WED-080

A LARGER COTYLEDON AREA AFTER GERMINATION IS A COMMON PHENOMENON IN HETEROTIC F1 HYBRIDS OF *ARABIDOPSIS THALIANA* AND CHINESE CABBAGE

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The hybrid progeny of selected parental lines exhibit enhanced performance relative to their parents in both plants and animals. This phenomenon is known as hybrid vigor or heterosis. F1 hybrid cultivars produce increased yield in maize and now hybrids are widely used other crops and vegetables. However the molecular mechanism of heterosis is still a mystery and there is no consensus model. *Arabidopsis thaliana* shows heterosis in progeny of particular combinations of parental lines such as Columbia (Col)-C24 and Landsberg erecta (Ler)-C24. Crucifer vegetables such as Chinese cabbage and turnip (*Brassica rapa*) and broccoli and cabbage (*Brassica oleracea*) are related to *A. thaliana*. In most crucifer vegetable cultivars F1 hybrid seed production systems are used. We studied the heterosis phenotype in Chinese cabbage (*B. rapa*) at early developmental stages (from germination to 14 days after sowing). In Chinese cabbage, heterosis in cotyledon area is seen a few days after sowing. As larger cell size and higher expression level of chloroplast-targeted genes has been reported in the C24xCol hybrids at 4 days after sowing, we examined cell size and expression level of chloroplast targeted genes in Chinese cabbage. We will discuss about similarity and differences in the heterosis phenotypes between the two species of Brassicaceae.

POS-TUE-081

THE DUF642 PROTEIN *AT2G41800* LOCALIZES TO PREPROPHASE BAND, PHRAGMOPLAST AND CELL WALL IN SYNCHRONIZED ROOT MERISTEM CELLS OF *ARABIDOPSIS THALIANA*

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In plants, cell wall is a complex and dynamic structure that is built by high molecular weight carbohydrates and proteins. Cell wall plays an important role during different stages of the plant life cycle such as cell growth and differentiation processes as well as protection against adverse environmental conditions, pathogens and/or herbivores. Cell wall proteins DUF642 constitute a highly conserved family of spermatophytes. The degree of diversification and the conservation of the family suggested that DUF642 proteins are key components in seed plant evolution. In particular, *At2g41800* is one of the most induced genes during M/G1 cell cycle phases and its protein has only been detected in cell wall proteomes of cell suspension cultures suggesting a role in cell wall formation during cell division. Callus induction of *Arabidopsis thaliana* roots from PROAt2g41800::At2g41800-GFP transgenic plants were used to visualize At2g41800 protein localization in cell wall. Synchronized root meristem cells were used to localize it during the last phases of cell cycle. At2g41800 was localized in the pre-prophase band and phragmoplast. Cell wall localization of the protein was observed during cytokinesis. The cell wall localization of At2g41800 protein in callus and root meristem cells was confirmed by electronic microscopy. Although the root growth of the transgenic line is not different, these plants have larger roots than wt seedlings during cold stress. An enhanced tolerance to chilling of the PROAt2g41800::At2g41800-GFP transgenic plants mediated by alteration in cell cycle is discussed.

POS-WED-082

LSM PROTEINS PROVIDE ACCURATE SPLICING AND DECAY OF SELECTED TRANSCRIPTS TO ENSURE NORMAL ARABIDOPSIS DEVELOPMENT

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In yeast and animals, Sm-like (LSM) proteins typically exist as heptameric complexes and are involved in different aspects of RNA metabolism. Eight LSM proteins, LSM1-8, are highly conserved and form two distinct heteroheptameric complexes, LSM1-7 and LSM2-8, that function in mRNA decay and splicing, respectively. A search of the *Arabidopsis thaliana* genome identifies eleven genes encoding proteins related to the eight conserved LSMs, the genes encoding the putative LSM1, LSM3 and LSM6 proteins being duplicated. The molecular and functional characterization of the Arabidopsis *LSM* gene family showed that the eleven *LSM* genes are active and encode proteins that are also organized in two different heptameric complexes. The complex LSM1-7 is cytoplasmic and is involved in P-body formation and mRNA decay by promoting decapping. The complex LSM2-8 is nuclear and is required for pre-mRNA splicing through U6 snRNA stabilization. These complexes are essential for the correct turnover and splicing of selected developmental-related mRNAs, and for the normal development of Arabidopsis. We propose that LSMs play a critical role in Arabidopsis development by ensuring the appropriate developmental-related gene expression through the control of mRNA splicing and decay.

POS-TUE-083

THE REGULATION OF GRAIN SIZE IN *BRACHYPODIUM DISTACHYON*

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While significant progress has been made towards understanding the regulation of seed size in Arabidopsis, relatively little is known about the regulation of grain size and development in cereals. Cereals have a very different grain composition to that of Arabidopsis and therefore the pathways involved in grain development are likely to vary. Brachypodium is an excellent model for analysis of the regulation of grain size in cereals as it has a small genome and the same basic grain structure as that of other cereals. We have developed a method to accurately screen grain size in Brachypodium through image analysis of grain samples using the CSIRO Workspace software. Using this method we have screened an EMS population for mutants with increased or decreased grain size. A number of candidate lines have been selected for sequencing and characterization of the mutated gene. To investigate conservation in grain development between model species, Brachypodium T-DNA lines for homologous genes known to affect seed size in Arabidopsis and cereals have been identified and phenotyped. Through these approaches we hope to identify and gain a better understanding of key genes which regulate grain size in cereals.

POS-WED-084

THE ROLE OF AUXIN AND CYTOKININS IN CAMBIUM DEVELOPMENT

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The vascular cambium is a secondary meristematic tissue which produces the majority of biomass on Earth, developing xylem centripetally and phloem centrifugally, the two main vascular tissue types. Phytohormones are central regulators of plant development and architecture. Previous studies showed that cambial activity is regulated by several different phytohormones, such as auxin and cytokinins, but the related molecular mechanisms are still mainly unknown. In the recent years the crosstalk between auxin and cytokinin signaling pathways has been implicated to be central regulatory mechanism in several different developmental processes: embryogenesis, root and shoot apical meristem activity, lateral root formation and primary root vascular patterning. The interaction of these two phytohormones takes place at the level of biosynthesis, signalling or transport. In the primary root vasculature, for example, auxin response is focused in the future xylem axis while cytokinin response occupies the procambial domain. These two domains are maintained by mutually inhibitory interaction at the transport and signaling level between these two phytohormones. During secondary development cytokinins and auxin has been shown to regulate cambium activity; however, the mechanism is still unknown. Our preliminary results indicate that both auxin and cytokinins are critical for the activation of the secondary growth in Arabidopsis thaliana root cambium. Apart from transiently overlapping domains during the activation stage, auxin and cytokinin signalling occupy spatially distinct domains in cambium. Our results show that mutations impairing auxin-cytokinin crosstalk severely disrupt the wild-type cambial organization. Together these results indicate that spatial distribution of auxin and cytokinin and their interaction at the signaling boundary is critical not only for cambial cell proliferation, but also for cambial patterning.

POS-TUE-085

COORDINATION OF AUXIN SENSING AND MERISTEM ACTIVITY DURING DEVELOPMENT

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Organ initiation requires the specification of a group of founder cells at the flanks of the shoot apical meristem and the creation of a functional boundary that separates the incipient primordia from the remainder of the meristem. Organ development is closely linked to the downregulation of class I KNOTTED1 LIKE HOMEOBOX (KNOX) genes and accumulation of auxin at sites of primordia initiation. JAGGED LATERAL ORGANS (JLO), a member of the LATERAL ORGAN BOUNDARY DOMAIN (LBD) gene family, is required for coordinated organ development in shoot and floral meristems. Loss of JLO function results in ectopic expression of the KNOX genes SHOOT MERISTEMLESS and BREVIPEDICELLUS (BP), indicating that JLO acts to restrict KNOX expression. JLO acts in a trimeric protein complex with ASYMMETRIC LEAVES2 (AS2), another LBD protein, and AS1 to suppress BP expression in lateral organs. In addition to its role in KNOX regulation, we identified a role for AS2 in regulating PINFORMED (PIN) expression and auxin transport from embryogenesis onwards together with JLO. We propose that different JLO and AS2 protein complexes, possibly also comprising other LBD proteins, coordinate auxin distribution and meristem function through the regulation of KNOX and PIN expression during Arabidopsis development. We will further show how JLO controls auxin perception, and address how JLO expression is regulated by feedback mechanisms.

POS-WED-086

CHARACTERIZATION OF GENES INVOLVED IN DEVELOPMENTAL CHANGES IN AGAVE TEQUILANA BY HETEROLOGOUS EXPRESSION IN *A. THALIANA*

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Our model species is *Agave tequilana*, used for the production of tequila and therefore an important crop plant for the Mexican economy. The transition from vegetative to reproductive growth signals the end of the plants life-cycle after 5-7 years and determines the time for harvesting. Little is known about the environmental or internal cues which lead to this developmental change and a deeper understanding of the process in order to control and regulate it would have enormous benefits for tequila production. Another developmental phenomenon of interest in Agave species is the formation of vegetative bulbils on the inflorescence when sexual reproduction is unsuccessful. Although bulbil formation occurs in other plant species, the mechanism employed by Agave species seems to be relatively unique, involving a complete reversal in the developmental state from reproductive to vegetative and the induction of thousands of new vegetative meristems on the pedicels of the inflorescence. Transcriptome analysis of *A. tequilana* has led to the identification of candidate genes putatively involved in the developmental changes described above, however the lack of genetic studies and transformation protocols, the long life span, size and monocarpic nature of *A. tequilana* make it an unwieldy experimental model, therefore we have chosen to functionally characterize Agave candidate genes in *A. thaliana* by both ectopic expression and complementation of available *A. thaliana* mutants. Data relating to involvement in developmental changes of several transcription factor families and members of the auxin efflux transporter family PIN will be presented.

POS-TUE-087

MOLECULAR CLONING AND FUNCTIONAL ANALYSIS OF THE EXPANSION GENE *PDEXT* IN POPULUS DELTOIDES

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Plant expansion genes have been found to play a vital part in cell enlargement and extension. The genus *Populus* contains species of economic and ecological values and no study have ever elucidated the function of expansion gene in a tree species. In this study, an expansion gene *PdEXT* was cloned from *Populus deltoides*, and its function was characterized by investigating its expression patterns and heteroexpression in a hybrid poplar (*Populus davidiana* × *Populus bolleana*) and *Arabidopsis thaliana*. *PdEXT* was specifically and highly expressed in the mature xylem and immature phloem of *P. deltoides*. Overexpression of *PdEXT* resulted in an increase in plant height and stem diameter, the number of leaves, and the size of xylem and phloem zone in poplar. Overexpression of *PdEXT* in *Arabidopsis* plants showed sturdier stem, wider leaves, increased height and postponed anthesis, as well as had ideal plant architecture. The results indicated that *PdEXT* might function as a positive regulator involved in promoting the growth of these organs by expanding cell wall in plants. This study sheds light on the molecular mechanism of *PdEXT* in growth and development of *P. deltoides*plants.

POS-WED-088

MOLECULAR GENETIC ANALYSIS OF *PDF3*, A TRANSCRIPTION FACTOR EXPRESSED SPECIFICALLY IN THE SHOOT EPIDERMIS

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Shoot apical meristems are composed of several cell layers. The outermost layer, L1, differentiates into the epidermis. Most of the genes specifically expressed in the epidermis contain a *cis*-regulatory sequence called the L1 box within their upstream promoter regions. In Arabidopsis, homeodomain transcription factors ATML1 and PDF2 regulate expression of epidermis-specific genes through binding to the L1 box of these genes and play a critical role in the differentiation of shoot epidermal cells. *pdf2-1 atml1-1* double mutants display severe defects in shoot epidermal cell differentiation. In order to identify further transcription factors involved in epidermal cell differentiation, we selected the transcription factor genes that show reduced levels of expression in *pdf2-1 atml1-1* and examined their expression patterns. Our study of transgenic plants carrying promoter-GUS reporter gene constructs revealed that a C2H2-type zinc finger protein is expressed specifically in the shoot epidermis. We named the gene *PDF3*. A T-DNA insertion mutant of *PDF3* showed wild-type phenotype but double mutants between *PDF3* and its homologous gene showed a seedling lethal phenotype. Function of these genes will be discussed.

POS-TUE-089

TRANSLATIONAL REGULATION OF THE *SAC51* MRNA BY THERMOSPERMINE

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The *acaulis5* (*acl5*) mutant of Arabidopsis shows severe dwarf phenotype associated with excess xylem differentiation. *ACL5* encodes thermospermine synthase. Thermospermine is a structural isomer of spermine. *SAC51* has been identified from a suppressor mutant of *acl5*, *sac51-d* that recovers the phenotype without thermospermine, and encodes a basic helix-loop-helix (bHLH) transcription factor. The *SAC51* mRNA contains five small upstream open reading frames (uORFs) in the 5' leader region and *sac51-d* has a nucleotide substitution in the 4th uORF, which enhances translation of the main ORF. While the *SAC51* promoter is not responsive to thermospermine, the *SAC51* 5' leader region fused to the CaMV 35S promoter up-regulates the GUS reporter activity in response to thermospermine, suggesting that thermospermine acts on the *SAC51* 5' leader sequence. In this study, we examined the effect of thermospermine on the translation of a reporter gene fused to the *SAC51* 5' leader sequence in yeasts. The fusion construct containing a drug resistant gene under a minimal promoter followed by the *SAC51* 5' leader sequence was integrated into the yeast genome. When applied by exogenous thermospermine or transformed with the *ACL5* gene, the yeast cells significantly improved the viability in the selective media. Our results suggest that the 5' leader of *SAC51* mRNA acts as a thermospermine-dependent riboswitch.

POS-WED-090

RELATION BETWEEN SEPAL BOUNDARY AND PETAL PRIMORDIUM IN *ARABIDOPSIS THALIANA*

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In a flower, four types of floral organs, sepal, petal, stamen, and carpel, arise in a concentric manner. Each concentric region is called a whorl, and floral homeotic genes establish the organ specification in this concentric pattern. Within a whorl, the position of floral organ is fixed relative to the shoot apical meristem: in *Arabidopsis thaliana* one sepal arises in the abaxial side, one in the adaxial, and two in lateral positions. Petal primordia form close to the sepal boundary in the second whorl. Several studies indicate that the organ positioning and primordia formation is controlled by unknown mechanism that is independent of the organ specification. To understand this organ-positioning mechanism, we investigated the spatial and temporal expression pattern of transcription factors expressing in the specified sites in floral buds: *PETAL LOSS (PTL)* in the sepal boundaries, and *RABBIT EARS (RBE)* in the petal primordia. By combined fluorescent proteins fused to these transcription factors, we found that sepal boundary and petal primordia are hardly overlapped, and that PTL protein *per se* is not the mobile signal. We also examined the function of RBE by investigating its interactors and ectopic expression analysis. We would propose how the positional signal transfers to the organ primordia formation.

POS-TUE-091

GENETIC SCREENING FOR NOVEL REGULATORY FACTORS OF SECONDARY CELL WALL FORMATION DURING XYLEM VESSEL CELL DIFFERENTIATION IN ARABIDOPSIS

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Xylem vessel cells make up the conductive systems to transport water and minerals from roots to other parts of the plant, and are characterized by the thick secondary cell walls (SCWs). Higher plants develop two types of xylem vessel cells: protoxylem type and metaxylem type with the helical and pitted patterns of SCW thickenings, respectively. Even though recent studies have revealed a number of key genes closely related to the differentiation of xylem vessel cell, little is known about the formation of SCWs. A NAC domain transcription factor VND7 is regarded to be a master regulator for xylem vessel cell differentiation. Overexpression of VND7 by the constitutive CaMV35S promoter ectopically induces transdifferentiation of various cells into vessel elements with helical SCWs like protoxylem vessels in Arabidopsis. Recently, we established a transgenic Arabidopsis line with the overexpression of VND7 fused with the VP16 transactivation domain and the glucocorticoid receptor (VND7-VP16-GR), in which most cells transdifferentiate into the "protoxylem-like vessel cells" with helical SCWs in a synchronous manner after dexamethasone treatment. In order to better understand molecular mechanisms underlying the formation of SCWs in vessel cells, a genetic screening of EMS-mutagenized VND7-VP16-GR line has been performed by microscopic observation of SCWs in ectopically induced vessel cells. Through the screening, we successfully isolated one mutant with highly disordered SCWs in the ectopic vessel cells. The mutant also showed unusual xylem vessel formation and dwarf phenotype under normal growth condition. Taken together with results of further phenotypic analysis on the mutant, we will discuss the formation of SCWs.

POS-WED-092

VND7-BINDING SEQUENCES REVEALED BY FLUORESCENCE CORRELATION SPECTROSCOPY

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The Arabidopsis thaliana NAC domain transcription factor, VASCULAR-RELATED NAC-DOMAIN7 (VND7), acts as a key regulator of xylem vessel differentiation. Our previous study revealed a large number of putative direct target genes of VND7, which encode a broad range of proteins, such as transcription factors, IRREGULAR XYLEM proteins and proteolytic enzymes including XYLEM CYSTEINE PROTASE 1 (XCP1). Moreover, at least two distinct regions in XCP1 promoter responsible for VND7 binding, X1E1 and X1E2, were identified by a promoter-deletion analysis and an electrophoretic mobility shift assay (EMSA). However, cis-elements for VND7-binding are still not fully understood. Therefore, in this study, we attempted to identify cis-elements of VND7 using a new technique with Fluorescence Correlation Spectroscopy (FCS) which allows us to characterize the molecular-molecular interaction quantitatively on a large scale. As a result, FSC successfully detected the binding between a fluorescence (TAMRA)-labeled X1E1 (TAMRA-X1E1) and NAC-domain of VND7 fused with maltose binding protein (MBP) (MBP-VND7(NAC)). In addition, the excess amount of fluorescence-free X1E1 completely competed with the TAMRA-X1E1, suggesting the FCS is comparable to the EMSA for the analysis of binding between cis-elements and transcription factors. We finally succeeded in narrowing down the binding sequence from 53 bp to 18 bp with the deletion- and point mutation-versions of fluorescence-free competitors. We are now searching cis-elements in the other direct target genes of VND7 and of other related NAC transcription factors associated with xylem cell differentiation, which will allow us to better understand the molecular mechanisms of vascular development.

POS-TUE-093

REGULATION OF PLANT EPIDERMAL CELL DIFFERENTIATION BY A TOMATO (*SOLANUM LYCOPERSICUM*) R3 MYB TRANSCRIPTION FACTOR

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In *Arabidopsis thaliana* the CPC-like MYB transcription factors [CAPRICE (CPC), TRIPTYCHON (TRY), ENHANCER OF TRY AND CPC 1, 2, 3/CPC-LIKE MYB 3 (ETC1, ETC2, ETC3/CPL3), TRICHOMELESS 1, 2/CPC-LIKE MYB 4 (TCL1, TCL2/CPL4)] and the bHLH transcription factors [GLABRA3 (GL3) and ENHANCER OF GLABRA 3 (EGL3)] are essential regulators of trichome and root-hair differentiation. We identified *TRY* and *GL3* homologous genes from the tomato genome and named them *SlTRY* and *SlGL3*, respectively. Phylogenic analyses revealed a close relationship between the tomato and Arabidopsis genes. Real-time reverse transcription PCR analyses showed that *SlTRY* and *SlGL3* were predominantly expressed in aerial parts of developing tomato. After transformation into Arabidopsis, *CPC::SlTRY* inhibited trichome formation and enhanced root-hair differentiation by strongly repressing *GL2* expression. On the other hand, *GL3::SlGL3* transformation did not show any obvious effect on trichome or non-hair cell differentiation. These results suggest that tomato and Arabidopsis partially use similar transcription factors for epidermal cell differentiation, and that a CPC-like R3 MYB may be a key common regulator of plant trichome and root-hair development.

POS-WED-094

PLOIDY-CELL SIZE RELATIONSHIP IS CONTEXT-DEPENDNET IN ARABIDOPSIS

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Ploidy level is positively reflected in cell size in fungi, plants and animals. This ploidy-dependent cell enlargement has been used as a tool for improvement of useful crops, fruits and flowers, through artificial polyploidization. In addition, it is widely believed that endoreduplication, the genome duplication process without intervening chromosome segregation in somatic cells, is also tightly linked to cell volume increase in Arabidopsis tissues. But we do not know why polyploidy affects the cell size in organisms. The most naïve explanation suggests that increased gene copy numbers result in an increase of proteins amounts that is finally reflected as an increase in cell volume. If this idea is correct, any strains, mutants, and transgenics should show the same fold-change in the cell size before and after polyploidization. Here we examined the above idea by tetraploidizing various mutants and transgenics of Arabidopsis having a wide range of cell size. Unexpectedly, we found that, the genetic background strongly affects the ratio of size between the original diploids and the induced tetraploids, namely, the ratio varied from 1.2 to 2.9 while it was ca. 1.8 in the wild type. In addition, by using a single-cell-level bio-imaging system, we found that cell volume does not necessarily refelect the ploidy level in some tissues and/or mutant lines. Taken all together, it is evident that the relationship between the ploidy level and cell size is not so stringently fixed, but is rather regulated in a context-dependent manner.

POS-TUE-095

DIFFERENTIAL PHASE SHIFT IN CLOCK CORE COMPONENTS ALTERS MULTIPLE HORMONE SIGNALING AND REDUCES COORDINATION FOR GROWTH AND LEAF MOVEMENT

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Plants grown under warm night/cold day (-DIF) show reduced elongation and leaf movement compared to plants grown under cold night/warm day (+DIF).). Using luciferase reporter plants we analysed expression of several clock genes (CAA1, LHY1, TOC1, Gi) in mature rosette plants under +/-DIF diurnal conditions. Results show that for plants grown under –DIF the phase of different clock genes is shifted compared to the phase under +DIF, but the magnitude and direction of this shift is different for each clock gene. This indicates that coordination of clock controlled processes could be altered under –DIF. A reduced coordination of clock controlled sequential steps required for cell elongation could therefore be the basis for compact plants under –DIF. Indeed the phase of three different clock regulated processes (leaf movement, ethylene emission, auxin signaling) all show differential phase shifts in response to -DIF. Dissection of the signaling components involved in petiole/hypocotyl elongation indicates that PIF4 activity is upstream of local auxin signaling which in turn is required for activation of petiole specific ACC synthase genes. The local production of ACC/ethylene in petioles correlates with leaf movement and petiole elongation. Under –DIF overall PIF4 activity and thus local auxin and ethylene synthesis is reduced. Moreover, also ethylene signaling capacity and ABA levels are reduced under -DIF. In contrast to the pif4 mutant, in the pif3 mutant the effect of –DIF on hypocotyl cell elongation cannot be complemented by auxin or ethylene, suggesting that PIF3 acts downstream of PIF4 in control of local cell elongation.

POS-WED-096

THE ARABIDOPSIS LEAF TRANSCRIPTOME CARTA: DYNAMIC LANDSCAPES OF MULTI-DIMENSIONAL TRANSCRIPTOME ALONG LIFESPAN

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Leaves harvest light energy and fix CO_2, being the only practical source of foods on earth. Leaves, during their lifespan, undergo developmental and physiological shifts, ending with senescence and death. Here, we used mRNA-seq and strand-specific total RNA-seq and smRNA-seq to provide the structure and dynamics of multi-dimensional (RNA type, organellar, and time dimensions) transcriptomes of *Arabidopsis* leaf during the entire lifespan. Novel findings from the *Arabidopsis* leaf transcriptome include utilization of chloroplast transcripts as a key constituent in leaf lifespan programs, global changes of non-coding as well as coding RNAs along lifespan, and generation of antisense transcripts in majority of chloroplast and mitochondrial genes. Importance of tRNA-derived smRNAs and trans-acting siRNAs in regulating leaf lifespan was also explored. Moreover, subsets of organellar transcriptomes were shown to be tightly coordinated along lifespan. This *Arabidopsis* leaf transcriptome provides a comprehensive resource for multi-dimensional understanding of functional and regulatory programs during lifespan.

POS-TUE-097

WOUND-INDUCED AUXIN FLUX TRIGGERS STEM CELL FATE TRANSITION FOR DE NOVO ROOT ORGANOGENESIS IN ARABIDOPSIS

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Plant cells, unlike most animal cells, have an ability to regenerate new tissues or organs from the detached ones. De novo root organogenesis from detached aerial organs is a common strategy for a plant to survive from accident physical damages, as regenerated adventitious roots could ensure the water supply for subsequent development of a new plant from the damaged part. It is known that wounding is the first and essential event for de novo root organogenesis; however, the molecular basis from wounding to root formation remains largely unclear. Using the Arabidopsis leaf explant system, we show that auxin is transported to the cambium cells at the wound site upon injury. The concentrated auxin then induces expression of the putative transcription factor genes WOX11 and WOX12, which may play roles in fate transition from cambium cells to root founder cells. Our data provide a framework of plant de novo root organogenesis, linking the wound-directed hormone action to the fate transition of stem cells.

POS-WED-098

REVEALING THE IDENTITY OF APPLE FRUIT FLESH

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Apple fruit flesh develops mostly from the accessory tissue fused to the ovary that contributes the formation of fruit core. The exact morphological origin of the accessory tissue has not been determined. The question remains as to whether it has an appendicular origin (resulting from fusion of the bases of floral appendages, i.e., sepals, petals and stamens), a receptacular origin (part of the floral axis), or a combination of both. Functional analysis studies of the floral organ genes *Pistillata* (Yao, et al. 2001, PNAS 98:1306-1311) and *Sepallata* (Ireland, et al. 2012, Plant J. Accepted Article, doi: 10.1111/tpj.12094) show that each change in identity and development of the sepal, petal and/or stamen is linked to a change in fruit development, suggesting an appendicular origin for the apple fruit flesh. Most interestingly, our recent study shows that flowers of transgenic apple plants over-expressing a miRNA gene completely lack sepal, petal and stamen tissues and have naked ovaries without any surrounding accessory tissue, and further supports the appendicular hypothesis.

POS-TUE-099

A RICE TCP TRANSCRIPTION FACTOR REGULATES TILLER FORMATION AND ROOT GROWTH

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Plant organ development requires controlled cell division and differentiation to achieve their final size and shape. We have identified TCP (Teosinte-branched/Cycloidea/PCNA) family transcription factors as highly up-regulated genes in the meristem region of rice high tillering d10 mutant. OsTCP6 encodes a nuclear-localized transcriptional activator. In situ hybridization and promoter analysis revealed that the OsTCP6 gene is expressed in SAM, crown root primordia, immature caryopses and pericycle of developing lateral roots. Ectopic expression of OsTCP6 in transgenic Arabidopsis increased leaf size and development, and cell cycle-related genes were up-regulated. In transgenic rice over-expressing TCP genes, tiller formation was activated, and root length and number were increased. Our current data implicates that OsTCP6 function as a positive player of organ growth and development. Supported by a grant PJ00951406.

POS-WED-100

SAC51 MEDIATES THERMOSPERMINE-DEPENDENT REPRESSION OF XYLEM PROLIFERATION

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Thermospermine is a structural isomer of spermine and is produced from spermidine by the action of thermospermine synthase, ACAULIS5 (ACL5). Since loss-of-function mutants of *ACL5* show excess proliferation of xylem vessels and the phenotype is enhanced by 2,4-D, thermospermine appears to have an opposite role to auxin in regulating xylem differentiation. A previous study has isolated suppressor mutants named sac that reverse the phenotype of *acl5. SAC51* encodes a bHLH-type transcription factor and contains five upstream open reading frames (uORFs) in the 5' leader region of the transcript. The *sac51-d* allele has a point mutation in the 4th uORF and has been shown to enhance translation of the main ORF, which may in turn down-regulate expression of a subset of genes involved in xylem differentiation. Heat shock-inducible expression of *SAC51* in transgenic *acl5* mutants result in the recovery of the phenotype in a heat shock-dependent manner. We have isolated a T-DNA knockout allele of *SAC51* and found that the mutant shows wild-type phenotype, suggesting functional redundancy between *SAC51*-like genes including *SACL1, SACL2,* and *SACL3*. We have also found that the *sac57-d* allele has a point mutation in the uORF homologous to the 4th uORF of *SAC51*. To identify genes whose expression is regulated by SAC51, the DEX-inducible system was used for SAC51 activation. The results will be presented.

POS-TUE-101

THE ROLE OF ARABIDOPSIS *HAWAIIAN SKIRT (HWS)* GENE IN FLORAL DEVELOPMENT AND ITS ORTHOLOGUE GENE *ERECT PANICLE3* (EP3) IN RICE

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Floral organ detachment takes place at a specifically programmed stage during development. However, sepals of the mutant *hawaiian skirt* (*hws-1*) fail to shed and are retained until silique desiccation. The mutant is a consequence of a 28bp deletion in the ORF of the F-box gene *At3g61590*, which is part of one of the three core subunits of the SCF complex of plant ubiquitin E3 ligases. Previous studies on *HWS* gene have showed that the non-shedding phenotype is a consequence of fused sepals rather than a failure of abscission zone cells to differentiate (González-Carranza et al. 2007). To dissect the role of HWS in regulating plant development, *hws-1* seeds were mutagenised with EMS and several suppressor lines of the *hws-1* phenotype have been identified. The gross mapping of 35.1, one of the suppressor lines, showed that the mutated gene is located at the bottom of Chr1, and a candidate gene *Apetala1* (*AP1, At1g69120*) was studied in detail. Sequence analysis showed a single nucleotide change in the *AP1* gene, from C to T located 107bp downstream of ATG in the CDS resulting an amino acid change from Ser to Phe in the conserved MADS box domain. Rice (*Oryza sativa*) has two putative orthologues of *HWS*, which are *Os01g47050* and *Os02g15950*. *ep3*, a Loss-of-function mutant in *Os02g15950*, showed a significant decrease on photosynthesis assimilation in our studies using gas exchange. González-Carranza, Z. H., U. Rompa, J. L. Peters, et al (2007). Hawaiian skirt: an F-box gene that regulates organ fusion and growth in Arabidopsis. Plant physiology 144(3): 1370-1382.

POS-WED-102

ACR4-DEPENDENT STEM CELL DIVISION IN ARABIDOPSIS ROOTS

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Throughout plant development, organogenesis and pattern formation depend on coordinated formative divisions to specify and maintain pools of stem cells. In the Arabidopsis root, the development of columella cells and lateral roots requires a tight coordination of asymmetric cell division and differentiation (De Smet and Beeckman, 2011, *Nature Reviews Molecular Cell Biology* 12:177-188).The receptor-like kinase ARABIDOPSIS CRINKLY4 (ACR4) has been identified as a key regulator of formative cell divisions both during columella stem cell divisions and lateral root initiation by regulating formative cell divisions and repressing excessive proliferation (De Smet *et al*, 2008, *Science* 322:594-597). Thus, the ACR4 function reveals a common mechanism of formative cell division control in the main root tip stem cell niche and during lateral root initiation.To get insight in the ACR4 signaling pathway, we performed a tandem affinity purification (TAP) study on the ACR4 intracellular domain. We identified several putative ACR4 interactors and new potential regulators of formative cell division and lateral root initiation. Our preliminary results revealed that *dhpr1*, *dhpr2* and *grf12* mutants showed significant changes in primary root length and emerged lateral root density. Furthermore, in *dhpr2* mutants, the differentiation of columella stem cell daughter cells was clearly delayed as more layers of undifferentiated columella cells were observed. In addition, mutants in subunits of PP2A complexes showed extra divisions in cortex and endodermis cell layers. These putative novel components of ACR4-dependent and -independent signaling pathways affecting stem cell division and root development will be discussed.

POS-TUE-103

MOLECULAR MECHANISMS OF ETHYLENE-AUXIN INTERACTIONS

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Fine tuning of the growth and development programs with the changes in the environment is a process of critical importance for plants that, due to their sessile lifestyle, cannot escape adverse environmental conditions. Plant hormones play a key role in the integration of signals triggered by endogenous and exogenous stimuli. To dissect the involvement of plant hormones in signal integration, the interaction between ethylene and auxin in the regulation of a highly plastic phenotype, root elongation, was chosen as a model. Our initial studies have uncovered an unexpected role of ethylene in the precise spatiotemporal regulation of auxin biosynthesis. Current work is unveiling an exciting new role of translational regulation as an additional point of interaction between ethylene and auxin.

POS-WED-104

HOW STRIGOLACTONES IMPACT ROOT SYSTEM ARCHITECTURE

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Strigolactones, known as regulators of shoot branching, also control root morphology: main root length, adventitious rooting, lateral root branching and root hair length are all affected by strigolactones. A detailed kinematic analysis (via mutant and pharmacological approaches) showed that the belowground effects of strigolactones are MAX2 dependent, transient and mainly apparent at younger stages during root development, and have also linked photomorphogenesis with root growth. To understand the underlying mechanisms of strigolactones in the below-ground organization of the plant body, we have thoroughly addressed the cross talk of strigolactones with other hormonal pathways (auxin, ethylene, jasmonates and cytokinin) important for a correct establishment of the root system. Our results suggest great importance of the cytokinin signaling pathway during lateral root inhibition by strigolactones. Despite its described importance for root hair formation, no clear role for ethylene could be discerned in strigolactone mediated lateral rooting, while a role for jasmonic acid was apparent. Additionally, to fully understand the *modus operandi* of strigolactones, different 'omics' approaches are undertaken in our lab. Firstly, we use the tandem affinity purification technique as a unique approach to identify signaling partners of MAX2, the presumed strigolactone receptor. Complementary, we have initiated a general and a highly specific transcriptomic strategy via RNAseq to uncover general transcriptome changes by strigolactones. Next to that, proteomic analyses are ongoing on mock and strigolactone treated roots to pinpoint the key effectors of strigolactone action in shaping the root. A progress overview of these combinatorial approaches, with emphasis on strigolactone signaling mechanisms, will also be presented here, altogether leading to a better understanding of the strigolactone signaling cascade.

POS-TUE-105

JASMONIC ACID SIGNALING IS LINKED TO AUXIN HOMEOSTASIS THROUGH THE MODULATION OF *YUCCA8* AND *YUCCA9* EXPRESSION

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Phytohormones regulate a wide array of developmental processes throughout the life cycle of plants. Over recent years, mounting evidence led to the widely accepted concept that phytohormone action is not the read-out of linear pathways, but determined by the extensive combinatorial activity of the signaling molecules and the integration of their signaling pathways, both in terms of regulating growth and development and in adapting to external stimuli. Recent work is beginning to shed light on the crosstalk of both nominally synergistically and antagonistically acting plant hormones. Here, we report that oxylipins contribute to the regulation of the expression of the Arabidopsis *YUC8* and *YUC9* genes. Similar to previously characterized YUC family members, we identify both enzymes as involved in local auxin biosynthesis, demonstrated by altered auxin contents and auxin-dependent phenotypes displayed by loss- and gain-of-function mutants. Expression data obtained by qPCR analysis and microscopic examination of reporter lines reveal an oxylipin-mediated regulation of *YUC8/9* expression that is dependent on the COI1-signal transduction pathway. The microscopic data indicate a functional overlap of the two analyzed auxin biosynthesis genes, but also point out specific functions for YUC8 and YUC9, which are in part related to different spatio-temporal expression pattern. In support of these findings, the analyzed yuc knockout mutants had lower free auxin contents and displayed a reduced response to oxylipins. In addition, analysis of *YUC8* and *YUC9* overexpression lines provided further evidence for the existence of a direct link between auxin content and ethylene biosynthesis. This work provides evidence of a molecular mechanism that links jasmonate signaling with auxin homeostasis and plant secondary growth.

POS-WED-106

BRASSINOSTEROIDS ATTENUATE ABA-INHIBITED EARLY SEEDLING DEVELOPMENT VIA BES1/TPL/HDA19-INDUCED EPIGENETIC SILENCING OF *ABI3*

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Seed germination and establishment of young seedlings are the most critical phases in plant life cycles. To tightly control these crucial processes, plants have evolved diverse hormonal signaling networks in which brassinosteroid (BR) is known to attenuate abscisic acid (ABA) responses; however, its regulatory mechanism remains elusive. Here, we reveal that BES1-initiated epigenetic silencing of *ABI3* is essential for BR-inhibited ABA signaling during early seedling development. BR-activated BES1 forms transcriptional repressor complexes with TPL via its EAR motif, and recruits Histone deacetylase HDA19. This event particularly facilitates Histone H3 deacetylation of *ABI3* chromatin leading to suppression of *ABI3* and its downstream *ABI5*, which results in lowered ABA sensitivity during early seedling development. Genetic and physiological evidences further support that BES1-mediated BR signaling pathways are delineated to ABI3/ABI5-mediated ABA signaling module. Taken together, we propose that BR-activated BES1-TPL-HDA19 repressor complex controls epigenetic silencing of *ABI3* in ABA response during early seedling development.

POS-TUE-107

COMPREHENSIVE DEGRADATION ANALYSES OF AUX/IAAS IN COMBINATION WITH SEVERAL AUXINS AND ALL TIR1/AFBS

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Auxin plays a key role in every aspect of plant growth and development. Auxin induces interaction of a TIR1 family receptor with an AUX/IAA protein, leading to degradation of the AUX/IAA. There are genes of 6 TIR1-like F-box proteins, 29 AUX/IAA proteins, and two types of auxins, IAA-type and Phenyl acetic acid (PAA)-type auxins in *Arabidopsis thaliana* . Therefore, there are many possible combinations between these three factors, which may contribute to complex auxin responses in plants. Here we aimed to clarify degradation efficiencies and auxin sensitivities of AUX/IAAs in all combinations. Yeast expressing TIR1 have been reported to degrade IAA17 in an auxin-dependent manner, therefore, we expanded this system. We constructed yeast strains expressing a pair of a receptor and a luciferase-fused AUX/IAA in all combinations, and measured luciferase activity after addition of different auxin molecular species. The results revealed that TIR1 and AFB2 could cause AUX/IAA degradation. Both IAA and PAA have activity to degrade AUX/IAA, with IAA much higher activity. All AUX/IAAs that lack domain II, which provide receptor-binding surface, were not degraded. It indicated that domain II is essential for auxin-induced degradation. Degradation efficiencies of AUX/IAAs that have domain II were variable. Finally, the orders of degradation efficiencies of most AUX/IAAs in the presence of TIR1 and in the presence of AFB2 were similar, but some AUX/IAAs showed specific preference for TIR1 or AFB2.

POS-WED-108

FUNCTIONAL DIVERGENCE BETWEEN THE STRIGOLACTONE AND KARRIKIN SIGNALLING COMPONENTS D14 AND KAI2 PRE-DATES THE EMERGENCE OF SEED PLANTS

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In many plants, the architecture of shoots and roots is regulated by strigolactones, endogenous phytohormones synthesised from carotenoid precursors. In Arabidopsis and rice, responses to strigolactones require the α/β hydrolase D14. The Arabidopsis paralogue of D14, AtKAI2, is required for normal seedling development and for responses to karrikins. Derived from burnt plant material and structurally similar to strigolactones, karrikins stimulate seed germination and promote seedling photomorphogenesis. Promoter-swap experiments indicate that AtKAI2 and AtD14 are non-interchangeable in Arabidopsis, raising the questions of when this functional distinction emerged, and whether one response system evolved from the other. The genomes of the liverwort *Marchantia polymorpha* - a non-vascular plant - and the lycophyte *Selaginella moellendorffii* - a vascular, non-seed plant - each encode two homologues within this α/β hydrolase family, but their precise phylogenetic relationships with AtKAI2 and AtD14 are ambiguous. To assess whether the functions of KAI2 and D14 are evolutionarily conserved, we have cross-complemented the Arabidopsis *Atkai2* and *Atd14* mutants with *Marchantia* and *Selaginella* homologues. Neither of the *Marchantia* homologues complemented the *Atkai2* mutant, whereas one of the *Selaginella* homologues could functionally substitute for AtKAI2. In contrast, none of the *Marchantia* or *Selaginella* homologues complemented the *Atd14* phenotype. Our results suggest that KAI2 signalling is conserved at least across vascular plants, whereas D14 function has evolved more recently. We conclude that the KAI2 signalling system is an evolutionarily ancestral trait that has been duplicated and modified in seed plants to transduce strigolactone signals that control shoot development.

POS-TUE-109

THE NAC-LIKE GENE GIBBERELLIN SUPPRESSING FACTOR CONTROLS PLANT GROWTH AND DEVELOPMENT BY REGULATING GIBBERELLIN METABOLISM PATHWAY IN *ARABIDOPSIS*

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GIBBERELLIN SUPPRESSING FACTOR (GSF) contained one highly conserved NAC domain in N-terminal region and a strong α-helical transmembrane motif (TM) in C-terminal region and has been predicted to be membrane-associated. In *GSF::GUS* plants, GUS activity was highly expressed in roots, cotyledon, shoot apical meristem and leaves. GSF has transactivation capacity based on yeast transcription activity assays. This result indicated that GSF acts as a transcriptional activator. YFP+GSF-TM (lacking a transmembrane domain) fusion proteins were accumulated in the nuclei of the Arabidopsis cells whereas the YFP+GSF fusion proteins were accumulated in the mitochondria membrane and absent in the nuclei. Transgenic plant ectopically expressing *GSF* was phenotypically indistinguishable from wild-type plants. By contrast, *35S::GSF-TM* exhibited phenotypic alterations such as dwarfism and curled leaves in transgenic Arabidopsis. These results revealed that GSF needs to be processing and releasing from the mitochondria and transported into the nucleus to perform its function. Further analysis indicated that the mutant phenotype in *35S::GSF-TM* was correlated with the up-regulation of gibberellin (GA) deactivation genes GA2-oxidase (*GA2ox*) that suppressed the GA activity, and down-regulation of GA biosynthesis genes GA20-oxidase (*GA20ox*) that increase the GA production in plants. In addition, levels of gibberellins in *35S::GSF-TM* plants were lower than that in wild type plants. External application of GA rescued the dwarfism in *35S::GSF-TM* plants. This result reveals that GSF does not affect the GA signaling pathway. Our data suggest a role for *GSF* in controlling multiple plant developments by suppressing the GA biosynthesis in Arabidopsis.

POS-WED-110

CHARACTERISATION OF CANDIDATE GLYCINE MAX SYMBIOSOME MEMBRANE PROTEINS

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The legume-rhizobium symbiosis has great importance to society as a source of high protein food and for enriching soil nitrogen. Legumes house their symbiotic bacteria, termed bacteroids, in a new plant organ called the nodule. Within infected nodule cells the bacteroids are encapsulated by the plant derived symbiosome membrane, which forms an interface for nutrient exchange. The primary exchange involves the supply of fixed nitrogen produced by the bacteroids, in return for energy and metabolites derived from the plant. Protein transporters embedded within the symbiosome membrane facilitate this exchange. My research focuses on characterising the role of putative symbiosome membrane proteins, discovered in the symbiosome membrane proteome of soybean (*Glycine max*). Two interesting candidates discovered in the proteome include Nodulin21, a potential iron transporter and PLAC8 a possible cadmium, zinc or calcium transporter. Both Nodulin 21 and PLAC8 are highly and specifically expressed in mature, nitrogen fixing nodules. A third candidate DUF588, is also specifically expressed in mature nodules but expression is isolated to infected cells only. Preliminary data from artificial microRNA knock down suggests a possible role in nodule development. I am currently confirming candidate localization to the symbiosome membrane using reporter gene fusions and functionally testing whether the potential metal transporters are involved in iron transport via yeast complementation.

POS-TUE-111

NATURAL VARIATION IN THE DEVELOPMENTAL CONSEQUENCES OF A LOSS OF CHLOROPLAST TRANSLATION IN *ARABIDOPSIS THALIANA*

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Interfering with chloroplast translation is typically more detrimental to growth and development in *Arabidopsis* than in *Brassica* or maize. This difference appears to reflect, in part, variation in the presence and functionality of a duplicated nuclear gene encoding a plastid-localized acetyl-CoA carboxylase (ACCase) required for fatty acid biosynthesis. In this study, we demonstrate that different accessions of *Arabidopsis thaliana* also vary in their ability to tolerate a loss of chloroplast translation. Two different approaches were pursed to block chloroplast translation: incorporation of spectinomycin into culture media used for seedling growth; and analysis of mutants defective in genes encoding chloroplast-localized ribosomal proteins. From an initial survey of 52 early-flowering accessions germinated on spectinomycin, 9 were chosen for further analysis: 3 tolerant accessions that produced albino rosettes; 3 sensitive accessions with at most rudimentary leaves; and 3 sensitive/intermediate accessions associated with knockouts of *EMB* genes encoding chloroplast-localized ribosomal proteins. When sensitive and tolerant accessions were crossed and responses of F2 plants analyzed on spectinomycin, results were in some cases consistent with a single locus conferring tolerance. Crosses were then performed between mutants defective in chloroplast translation and representatives of tolerant and sensitive accessions. This resulted in the identification of a suppressor locus that partially rescues mutant seeds and maps to a region on chromosome 1 that includes *ACC2*. Additional modifiers that support further embryo development were also found. Surprisingly, qPCR experiments revealed that *ACC2* is not over-expressed in seedlings of the tolerant accession examined. Whether *ACC2* activation in transgenic plants completely rescues mutant embryos remains to be determined. This work highlights the importance of evaluating accession-specific differences in mutant phenotypes in *Arabidopsis*.

POS-WED-112

CRYSTAL STRUCTURE OF RICE IMPORTIN-α AND STRUCTURAL BASIS OF ITS INTERACTION WITH PLANT-SPECIFIC NUCLEAR LOCALIZATION SIGNALS

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In the classical nucleocytoplasmic import pathway, nuclear localization signals (NLSs) in cargo proteins are recognized by the import receptor importin-α. Importin-α has two separate NLS binding sites (the major and the minor site), both of which recognize positively charged amino acid clusters in NLSs. Little is known about the molecular basis of the unique features of the classical nuclear import pathway in plants. We determined the crystal structure of rice (Oryza sativa) importin-α1a at 2-Å resolution. The structure reveals that the autoinhibitory mechanism mediated by the importin-β binding domain of importin-α operates in plants, with NLS-mimicking sequences binding to both minor and major NLS binding sites. Consistent with yeast and mammalian proteins, rice importin-α binds the prototypical NLS from simian virus 40 large T-antigen preferentially at the major NLS binding site. We show that two NLSs, previously described as plant specific, bind to and are functional with plant, mammalian, and yeast importin-α proteins but interact with rice importin-α more strongly. The crystal structures of their complexes with rice importin-α show that they bind to the minor NLS binding site. By contrast, the crystal structures of their complexes with mouse (Mus musculus) importin-α show preferential binding to the major NLS binding site. Our results reveal the molecular basis of a number of features of the classical nuclear transport pathway specific to plants.

POS-TUE-113

AN ESSENTIAL ROLE FOR ARABIDOPSIS TRS33, A COMPONENT OF TRAPP IN CELL GROWTH AND ORGANIZATION IN PLANT APICAL MERISTEMS

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TRAPP (trafficking protein particle) is a multimeric protein complex involved in the regulation of membrane trafficking. TRS33 is a component of TRAPP. In yeast it is non-essential for cell growth and viability. A single homologue of the *Trs33* gene named *AtTRS33* was found in the *Arabidopsis* genome based on sequence similarity. Analysis of a T-DNA insertion line indicates that, unlike its yeast homologue, AtTRS33 is essential for cell development in *Arabidopsis*. Perturbation of the *AtTRS33* expression by either RNA interference or over-expression leads to apical meristematic cell growth defects and reduced fertility, indicating that AtTRS33 plays an important role in cell growth and organization in plant apical meristems. Using pWOX5::GFP, a cell marker line that identifies the quiescent center, we revealed that cell files in root tips of AtTRS33-RNAi lines are mis-organized. Analysis of auxin response and PIN1 and PIN2 localization indicates that auxin response in root tip cells is altered and there is a severe disruption in polar localization of PIN1-GFP, but to a lesser extent, in polar distribution of PIN2-GFP in the root tips.

POS-WED-114

ASYMMETRIC DISTRIBUTION OF ATSWEET SUCROSE EFFLUXERS IN PHLOEM PARENCHYMA TRANSFER CELLS OF ARABIDOPSIS VEINS

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Members of the SWEET family of sugar transporters were recently identified as sucrose effluxers responsible for unloading symplasmically-delivered sucrose into the apoplasm for subsequent uptake into cells of the sieve element/companion cell (SE/CC) complex. Analysis of transgenic Arabidopsis plants expressing an AtSWEET11-eGFP fusion showed that AtSWEET11 is expressed in leaf tissue specifically in cells suggested to be phloem parenchyma transfer cells (Chen et al 2012 *Science* 335, 207-211). In Arabidopsis, these phloem parenchyma transfer cells develop highly-localized wall ingrowths adjacent to neighbouring cells of the SE/CC complex. This anatomy suggests a mechanism to achieve highly-targeted or localized efflux of sucrose from phloem parenchyma directed to the SE/CC complex. Using confocal microscopy, we investigated whether AtSWEET11 is distributed uniformly around the plasma membrane of phloem parenchyma transfer cells, or is restricted to regions of the plasma membrane associated with wall ingrowths. Confocal microscopy revealed that AtSWEET11-eGFP fluorescence appears to be concentrated into discrete, linear domains within phloem parenchyma cells that appeared to be adjacent to cells of the SE/CC complex. Time-lapse imaging of sepal veins detected movements of small fluorescent vesicles, consistent with the possibility that targeted exocytosis of AtSWEET11-containing vesicles may play a role in achieving the localized distribution of AtSWEET11 effluxers. This observation may provide new insight into control of sucrose efflux from phloem parenchyma transfer cells as a key step in active, apoplasmic phloem loading in Arabidopsis. Further experimentation revealing the distribution of AtSWEET11 in phloem parenchyma transfer cells of Arabidopsis veins will be reported.

POS-TUE-115

THE SENSITIVITY OF ACTIN KNOCKOUT MUTANTS TO MICROFILAMENT DISRUPTION WITH LATRUNCULIN B DEPENDS ON TOTAL ACTIN EXPRESSION LEVELS

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The primary root of *Arabidopsis* expresses three different actin genes (*ACT2*, *ACT7*, *ACT8*). *ACT2* and *ACT7* differ by 7 amino acids out of 345 while the weakly expressed *ACT8* differs by a single amino acid from *ACT2*. It is not yet clear whether these actin isoforms have specific roles within root cells, nor whether their polymerisation into microfilaments differs. To investigate this, we quantified aspects of root growth in *act2-1* and *act7-1* T-DNA knockout lines subject to increasing concentrations of the microfilament disrupting drug latrunculin B. In *act2-1*, root elongation was inhibited and radial expansion promoted at latrunculin concentration well below those that modulate wild-type growth. Root hair elongation was also strongly inhibited in this mutant. By contrast, root hair growth in *act7-1* was less sensitive to latrunculin than wild-type plants even though root elongation was greatly inhibited. Radial expansion was also promoted at much lower latrunculin concentrations than either *act2-1* or wild-type plants. The *act7-1* mutant was also sensitised to microtubule disruption with oryzalin suggesting interactions between microfilaments and microtubules. The variations in the actin microfilament cytoskeletons of these different lines in response to latrunculin are being investigated through immunofluorescence and with GFP-fABD2 expressing lines. We also conducted similar growth experiments in an ACT7P:ACT2 *act7-4* line where the *act7-4* mutation has been complemented by the expression of ACT2 under the ACT7 promoter. Because these plants are more resistant to latrunculin than wild-type plants, we conclude that varying sensitivities to latrunculin are not isoform dependent but instead are likely due to the overall concentration of actin within the different cell lines and types.

POS-WED-116

MOLECULAR MECHANISMS OF PVC-VACUOLE TRAFFICKING IN PLANT CELLS

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There are two main vacuolar trafficking pathways in the plant endomembrane system: the secretory and the endocytic pathways, merging in the trans-Golgi network (TGN) or early endosome. Rab proteins are members of the Ras superfamily of small GTP binding proteins, which play important roles in mediating vesicle trafficking. The aim of this study is to use *Arabidopsis* Rab proteins as probes to characterize the plant vacuolar trafficking from prevacuolar compartment (PVC) to vacuoles by using a combination of live cell imaging and biochemical approaches. We first tested several candidates of XFP-tagged Rab proteins for their subcellular localization and possible functions in vacuolar trafficking via transient expression in *Arabidopsis* protoplasts and stable expression in transgenic *Arabidopsis* plants. Preliminary results showed that Rab5 and Rab7 families localized on PVC and tonoplast respectively that may function in mediating PVC to vacuole trafficking in plants. Studies using dexamethasone (Dex)-induced transgenic plants expressing wild-type (WT) or dominant-negative (DN) forms of Rab5 or Rab7 homologs individually show that these proteins are essential for PVC to vacuole trafficking and vacuole biogenesis in plants. Further studies using pull down and proteomic analysis of transgenic plants will be carried out to identify their interacting partners for functional characterization. Here we will present an update about this study. Supported by grants from the Research Grants Council of Hong Kong (GRF & CRF) and CUHK Schemes.

POS-TUE-117

ROLE OF SODIUM-PROTON ANTIPORTERS *NHX5* AND *NHX6* IN THE ARABIDOPSIS SECRETORY PATHWAY

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Transmembrane sodium-proton antiporters have been shown to be associated with diverse physiological processes such as cell expansion and differentiation, salt tolerance and vesicle trafficking. Two closely related Na^+-H^+ exchangers (NHX), *AtNHX5* and *AtNHX6*, act redundantly to regulate cellular ion homeostasis and plant development in *Arabidopsis*. There is increasing evidence that *AtNHX5* and *AtNHX6* are involved in cellular trafficking to the vacuole. During maturation, the seeds of higher plants store long-term reserves of nitrogen in the form of storage proteins, which accumulate in Protein Storage Vacuoles (PSVs). These seed storage proteins (SSPs) provide an excellent tool for identifying genes associated with the vacuolar trafficking pathway in plant cells. SSPs are co-translationally inserted into the ER lumen and undergo post-translational cleavage *en route* to the PSV. The PSVs of *nhx5 nhx6* double mutant embryos were examined by confocal microscopy and found to be smaller and more numerous than in wild type, and *nhx5 nhx6* seeds abnormally accumulate the precursor form of the major storage proteins, indicating that these proteins play a role in vacuolar trafficking of endogenous soluble cargo. To better understand the role of NHX5 and NHX6 in the secretory pathway, the secretome of *nhx5 nhx6* double mutant seedlings was analysed and compared to wildtype plants. Proteins present in secreted media were identified by mass spectrometry; those found to be "mis-directed" in the double mutant were subject to further analysis. This information may give some insight into the specific pathways which are being affected by endosomal NHX proteins.

POS-WED-118

A ROLE FOR THE MITOCHONDRIAL REDOX-RELATED LEA PROTEIN (SAG21/ATLEA5) IN THE REGULATION OF PLANT GROWTH AND STRESS TOLERANCE

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We first isolated SAG21/AtLEA5, which belongs to the late embryogenesis-associated (LEA) protein family, using a functional cloning approach to identify novel plant genes involved in oxidative stress tolerance. We found that the expression of SAG21/AtLEA5, conferred tolerance to pro-oxidants in the oxidant-sensitive yeast mutant, Δyap11. Constitutive over expression (OEX) of SAG21/AtLEA5, in Arabidopsis thaliana increased root and shoot biomass under optimal and oxidative stress conditions1, whereas these parameters were decreased in antisense (AS) lines 2. Lateral root formation and root hair expansion were severely impaired in AS lines2. The expression of SAG21/AtLEA5, is enhanced by pro-oxidants, biotic and abiotic stresses and plant hormones such as abscisic acid, salicylic acid, ethylene and methyl jasmonate. These data suggest that this mitochondrial protein plays an important role in the control of plant development and biotic and abiotic stress responses. 1. Mowla et al. (2006) Plant J. 48, 743–756 2. Mohd Salleh et al (2012) Plant Cell & Env. 35, 418–429.

POS-TUE-119

CHARACTERISATION OF TWO GLYCOSYL TRANSFERASES INVOLVED IN ARABINOGALACTAN-PROTEIN BIOSYNTHESIS

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Arabinogalactan-proteins (AGPs) are cell surface proteoglycans implicated in many aspects of plant development, including cell expansion and division. AGP biosynthesis is complex and involves numerous post-translational modifications including heavy glycosylation of protein backbones. The enzymes involved in catalysing the formation of glycosidic bonds are known as glycosyl transferases (GTs). There are 43 different classes of GTs in *Arabidopsis* based on their predicted catalytic specificity. The GT 31 family members are thought to be involved in the biosynthesis of the AGP β-(1,3)-galactan backbone. Here, we describe the characterisation of two GT 31 family members, *At*1g74800 (*GALT2*) and *At*1g77810 (*GALT9*) and their involvement in AGP biosynthesis. Both GALT2 and GALT9 are localised in the Golgi, as predicted for GTs involved in the formation of the β-(1,3)-galactan, and are expressed in vegetative and reproductive tissues including pollen grains. Plants which lack *GALT2* or *GALT9* function have reduced AGP levels in flowers (~10%). Strikingly, plants that over-express *GALT2* have significantly higher AGP content in leaves (50% more than wild type) and display a swollen trichome socket cell phenotype. This result is consistent with the role of AGPs in cell expansion. The characterisation of these lines, as well as other data related to *GALT* function, will be discussed with respect to their involvement in AGP synthesis.

POS-WED-120

CONSTRUCTING THE SCAFFOLD OF THE PROTEIN-BUILDING MACHINERY: IDENTIFICATION OF A PENTATRICOPEPTIDE REPEAT PROTEIN INVOLVED IN CHLOROPLAST RIBOSOMAL RNA BIOGENESIS

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As chloroplasts are derived from a prokaryotic ancestor, they contain eubacterial 70S-type ribosomes. The ribosomal RNA component is encoded by an operon in the chloroplast genome which shows absolute conservation amongst higher plant chloroplasts. Maturation of the rRNA precursor transcript is carried out by ribonucleases, but the precise mechanisms involved remain to be discovered. One likely feature is the involvement of RNA-binding proteins, such as pentatricopeptide repeat (PPR) proteins, in defining precursor ends by blocking nucleolytic activity. We have identified a PPR protein, SOT1, which is involved in the biogenesis of the 23S rRNA precursor. Analysis of rRNA species in the sot1 mutant by Northern blotting indicates a disruption in chloroplast 23S rRNA biogenesis. Examination of the 5' region of the 23S rRNA precursor, using 5' RACE (rapid amplification of cDNA ends), indicates that the 23S rRNA is missing its 5' extension which correlates with the reduced size of the largest precursor identified in the 23S RNA blots. Using the "PPR code" (1) to predict the binding site for SOT1 resulted in the discovery that the sequence predicted aligns with the 5' region of the 23S rRNA precursor identified by RACE. Moreover, the predicted binding site aligns with a putative RNA-binding protein footprint recently published (2). Taken together, these observations strongly suggest that SOT1 binds to the 5' region of the 23S rRNA precursor. Experiments to confirm direct binding of SOT1 to this specific sequence are currently underway. (1) Barkan et al, 2012, PLoS Genet, 8:e1002910 (2) Ruwe & Schmitz-Linneweber, 2012, Nucleic Acids Res, 7:3106.

POS-TUE-121

SHAPER - A COMPUTATIONAL TOOL FOR AUTOMATIC QUANTITATIVE ANALYSIS OF CELL SHAPE AND ITS USE IN A CHEMICAL GENETICS APPROACH IN PLANTS

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Leaf epidermal pavement cells from plants have been attracting interest in recent years as a model system for cell shape formation in plants. Plant cell shape, seen as an integrative output, is of considerable interest in various fields, such as cell wall research, cytoskeleton dynamics and biomechanics. A major limiting factor in the study of cell shape has been the availability of computational methods to 1) numerically describe cell shape and 2) conduct unbiased statistical and/or correlative analysis on populations of shapes. We present ShapeR, a comprehensive software package for quantitative, size-independent statistical comparison of shapes based on elliptic Fourier analysis. ShapeR extends the elliptic Fourier analysis technique to enable the statistical analysis of complex, irregular shapes. This is facilitated by 1) an implementation of Generalised Procrustes Alignment to automatically align multiple shapes and 2) the extraction of invariant shape components, which are not influenced by shape orientation. Subtle differences in shape are detected through the use of linear discriminant analysis, a data mining method which maximises the separation between pre-defined groups. The differences between groups of shapes are then statistically tested using robust, non-parametric statistical methods. We have incorporated ShapeR into an automated pipeline for high-throughput screening of plants for defects in pavement cell shape. This incorporates plant growth in multi-well plates, robotised microscopy for high-throughput imaging and automated image processing for unsupervised extraction and statistical analysis of cell shapes. We have successfully used this set up to conduct a bioassay, screening a large library of diverse small molecules with the aim of discovering new drugs which affect cellular processes underlying cell shape formation, eg. cytoskeleton dynamics and cell wall deposition.

POS-WED-122

A NOVEL MECHANISM OF GOLGI RETENTION FOR INTEGRAL MEMBRANE PROTEINS

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Endomembrane Proteins (EMPs) are polytopic integral membrane proteins with a large lumenal N-terminus, nine transmembrane domains (TMD) and a short cytoplasmic tail (CT). We have recently demonstrated that the CT of the Golgi-localized Arabidopsis EMP12 contains both ER export (FVY) and Golgi-retention (KXE/D) signals for its proper Golgi targeting and localization in Arabidopsis cells (Plant Cell 24:2086-2104). These two motifs were also shown to interact with COPII and COPI subunits respectively. Our recent and on-going studies focus on understanding the underlying mechanisms of Golgi retention of memebrane proteins regulated by this novel KXE/D motif in eukaryotic cells including yeast, plants and animals using a combination of cellular, molecular, biochemical and genetic approaches. Here we will present an update on the research progress of this study. Supported by grants from the Research Grants Council of Hong Kong (GRF & CRF) and CUHK Schemes.

POS-TUE-123

UNDERSTANDING OF LEAF SENESCENCE-ASSOCIATED NAC TRANSCRIPTIONAL REGULATORY NETWORK IN ARABIDOPSIS

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Leaf senescence is an essential developmental process that involves extensive reprogramming and modulation of gene expression to maximize plant fitness. The onset and progression of leaf senescence is tightly controlled by transcription factors (TFs), of which NAC (for NAM, ATAF, and CUC) TFs are crucial in *Arabidopsis*. The *Arabidopsis* genome contains more than 100 *NAC* genes, and genetic and global transcriptome studies have shown that NAC TFs are involved in regulating stress responses as well as developmental processes, including leaf senescence. However, there is a little information on a system-level understanding of the NAC transcriptional regulatory network underlying leaf senescence. Here, we focused on 29 *NAC* genes which are up-regulated in senescent leaf. Gene regulatory networks, protein-protein interaction networks, and genetic interaction networks of senescence-associated *NAC* genes were systemically and comprehensively analyzed. Through these results, we identified the several central modulators in NAC TF networks and generated transcriptional cascade by inter-regulation or auto-regulation. This will be the first step in constructing integrated network for TF family in leaf senescence, and furthermore, in understanding evolutional principles of functional network modules to increase adaptability of a system and to allow rapid/robust informational flow though network.

POS-WED-124

UNDERSTANDING HOW RNA EDITING FACTORS RECOGNIZE THEIR RNA TARGETS

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Pentatricopeptide repeat (PPR) proteins bind RNA and determine a wide range of RNA processing events, such as stability, splicing and editing. RNA editing is essential for many transcripts in plant organelles and alters specific sites in the RNA sequence. PPR motifs are responsible for the RNA specificity of the editing reaction. The aim of this project is to obtain the knowledge, by studying editing PPR proteins in terrestrial plants, to design and construct proteins capable of binding user-defined RNA sequences. The large quantity of genetic data on plant PPR proteins and the availability of mutant seed stocks make plants the best model system for these studies, but since PPR proteins exist in all eukaryotes, future biotechnological potential applies to a wide range of organisms. We recently published an amino acid code of how PPR motifs recognize RNA bases. Using this code, we can now test the RNA specificity both in vivo and in vitro. By systematically mutate the RNA interacting amino acids and the target RNA for CLB19, which edits rpoA and clpP in plastids, we can confirm the binding site and target specificity. In addition, synthetic proteins have been created where the PPR tract from another editing factor, YS1, have replaced the PPR tract of CLB19. The synthetic proteins still retains the target peptide and the C-terminal domains of CLB19, ensuring a correct localization in vivo. Changing different combinations of the RNA interacting amino acids in the inserted PPR tract, we can investigate if it is possible to change unrelated PPR proteins to bind user-defined target sequences. How editing factors recognise their target sequences will be further discussed.

POS-TUE-125

FUNCTIONAL CHARACTERIZATION OF MITOCHONDRIAL OUTER MEMBRANE PROTEINS IN *ARABIDOPSIS THALIANA*

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The mitochondrial proteome consists of about a thousand proteins. While the mitochondrial matrix and inner membrane proteome have been well described, little is known about the protein composition of the inter membrane space and the mitochondrial outer membrane. The mitochondrial outer membrane acts as an interface between the cell and the other subcompartments and is responsible for processes such as sensing the intracellular signals and uptake of various macromolecules such as protein precursors, nucleic acids and metabolites. In order to investigate the mitochondrial outer membrane proteome of Arabidopsis thaliana, our laboratory has conducted a study, which identified 42 outer membrane proteins, doubling the number of proteins from previous studies. Some of these were shown to be dual targeted making up to 50 novel outer membrane proteins. In order to characterize the function of these proteins we used different approaches such as the in vitro uptake assays of proteins and nucleic acids and the fluorescent protein sublocalization on a collection of knockout and overexpressor lines in the outer membrane proteins. This poster aims to present the putative function of some of these proteins.

POS-WED-126

A PUTATIVE SOLUBLE TRAFFICKING COMPONENT MIGHT PLAY A NOVEL ROLE AS A MEDIATOR IN POST-GOLGI TRAFFICKING OF ION EXCHANGERS UNDER SALINITY STRESS IN *ARABIDOPSIS THALIANA*

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Resistance to abiotic stresses are supported by membrane trafficking from the trans-Golgi network (TGN), but the molecular mechanisms that promote membrane trafficking from the TGN under salinity stress are poorly defined in plant cells. Here we study a putative soluble trafficking component in Arabidopsis is thought to regulate the post-Golgi trafficking that is crucial for resistance to the salinity stress. Disruption of *AtSTC* which is co-localized with *AtVTI11* results in hyper-sensitivity to salinity stress in germination and impaired accumulation of proanthocyanidins in vacuole. Furthermore *AtSTC* loss-of-function leads to the increase of vcuolar pH. To investigate further, we plan some analyses. Together with these results, we suggest that *AtSTC* might play a novel role as a mediator in post-Golgi trafficking of ion exchangers.

POS-TUE-127

A GT47 FAMILY GLYCOSYL TRANSFERASE FROM *NICOTIANA* POLLEN MEDIATES THE SYNTHESIS OF (1,5)-α-L-ARABINAN WHEN EXPRESSED IN *ARABIDOPSIS THALIANA*

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Tobacco pollen tubes are used to study plant cell wall synthesis and assembly because of their relatively simple walls that are mainly composed of the polysaccharides (1,3)-β-D-glucan (callose) and (1,5)-α-L-arabinan, along with lesser amounts of cellulose, homogalacturonan and xyloglucan. As part of an RNA-Seq analysis several putative GT cDNAs were identified in *Nicotiana alata* pollen grains. One of these (*NaARAT1*) encodes a type II membrane protein of 489 amino acids with high sequence similarity to *At*ARAD1, a presumed (1,5)-α-arabinosyltransferase (AraT) from *Arabidopsis* belonging to CAZy glycosyltransferase family 47. Here we show that the *Na*ARAT1 promoter directs GUS expression in vegetative tissues of *Arabidopsis* as well as in pollen grains, and that a fluorescently tagged version of *Na*ARAT1, when transiently expressed in tobacco pollen tubes and leaves, is targeted to the Golgi apparatus, a location that is consistent with a presumed role for enzymes involved in arabinan synthesis. More importantly, *Arabidopsis* plants constitutively expressing *NaARAT1* have increased levels of arabinan in their cell walls, consistent with the identity of *Na*ARAT1 as a (1,5)-α-L-arabinan transferase.

POS-WED-128

THE MOLECULAR MECHANISM OF ELF4 TO REGULATE GI SUB-NUCLEAR DISTRIBUTION

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Daily rhythms in the plant circadian system are generated by multiple intrlocked transcription/translational loops and also by spatial regulations such as nuclear translocation. GIGANTEA (GI), one of the key clock components iN Arabidopsis, makes distinctive nuclear bodies like other nuclear-localized circadian regulators. We recently characterized GI sub-nuclear compartmentalization and identified unexpected dynamic changes under diurnal contions. We further identified EARLY FLOWERING 4 (ELF4) as a regulator of GI nuclear distribution through a physical interaction. ELF4 sequesters GI fromthe nucleoplasm, where GI binds the promoter of CONTANS (CO), to discrete nuclear bodies. We suggest that the subnuclear compartmentalization of GI by ELF4 contributes to the regulation of photoperiodic flowering. We are now trying to unravel the molecular mechanism how ELF4 regulates GI sub-sub nuclearcompartmentalization through interdisciplinary approaches, for example, biochemicaltechniques and super-resolution imaging tools.

POS-TUE-129

CHARACTERISING *ARABIDOPSIS* NITRILASE FAMILY AND 14-3-3 PROTEIN INTERACTIONS

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Arabidopsis thaliana has four nitrilase isoforms with disputed biological roles. It was first thought that these enzymes are involved in auxin biosynthesis. However, enzymatic studies indicated that *Arabidopsis* nitrilases are most likely not involved in the major auxin synthetic pathway. Instead, a new role in detoxification of glucosinolate-derived metabolites was suggested. An interesting aspect of nitrilases is their change of subcellular localisation upon chemical or physical stresses applied to plants. For example, treating cells expressing a nitrilase-GFP fusion protein with a nitrile herbicide or metabolites derived from glucosinolates induces re-localisation of a nitrilase from the cytosol to aggregate-like structures which appear to align in a network pattern. These results may indicate that nitrilases may be involved in stress response and possibly defence. Using Bimolecular Fluorescence Complementation, we showed that all four nitrilase isoforms have the ability to interact with 14-3-3 proteins in planta. 14-3-3 proteins are regulatory proteins which regulate client proteins' activity, localisation or interaction with other proteins. 14-3-3 proteins are implicated in hormone and stress responses in plants. Thus, it is possible that 14-3-3 bind to nitrilases to regulate their activity or localisation in accordance with cellular requirements under such conditions. Currently, we are characterising these interactions with the aim to determine their biological role with a focus on their cellular distribution upon stress treatments.

POS-WED-130

UNDERSTANDING THE GENETIC BASIS FOR THE STRUCTURAL DIVERSITY OF THE HEMICELLULOSE XYLOGLUCAN

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All plant cells are encased in a cell wall, a structurally complex and divers composite material. Understanding its synthesis has been an important issue for using this abundant renewable resource in industrial applications. One of the walls components, xyloglucan, the major hemicellulose present in the primary cell walls of most plant tissues, is important for structural organization of the wall and regulation of growth and development. Its biosynthesis requires glucan synthases (CSLCs) to form the glucan backbone and multiple different types of glycosyltransferases to decorate the glucan chain. The transferases required involve xyloglucan xylosyltransferases (XXTs) as a primary substituent as well as galactosyltransferases and fucosyltransferases, which would elongate diverse xyloglucan side chains. However, the fine structure of xyloglucan varies in different plant species and the functional significance and the genetic basis of these differences are not understood. Most vascular seed-bearing plants such as *Arabidopsis thaliana* and Nasturtium (*Tropaeolum majus*) synthesize triple substituted backbone oligomers, but plants in the Solanaceae family are made of double substituted backbone oligomers. By transforming a number of different functional and non-functional XTTs from various species (i.e. Nasturtium, Solanum and Arabidodpsis) in double xxt1/2 and triple xxt1/2/5 Arabidopsis knockouts, the changes in xyloglucan structure has been assessed and will help unravel the basis between the different types of xyloglucan subunits and ascertain the responsible gene(s) and their exact function. Subsequently, plants harboring these different structures can be assessed for various traits such as susceptibility to pathogens and/or mechanical strength.

POS-TUE-131

DOES EDITING OF THE *RPOC1* TRANSCRIPT REGULATE ACTIVITY OF THE PLASTID-ENCODED RNA POLYMERASE?

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RNA editing in higher plants represents a crucial step in maturation of organellar RNA that involves cytosine to uracil conversions at specific sites of the transcript. Out of 450 pentatricopeptide repeat (PPR) proteins identified in the Arabidopsis genome, 87 contain a C-terminal amino acid triplet (Asp(D)-Tyr(Y)-Trp(W)) referred to as the DYW domain, known to be involved in RNA editing. The *flavodentata* (*flv*) mutant was originally discovered in a collection of Arabidopsis X-ray induced mutants as a plant with serrated white leaf margins at the early developmental stage. Map-based cloning determined that the *FLV* gene encodes a plastid located PPR-DYW protein. A high resolution melting screen of mutants defective in editing identified FLV as an editing factor of the *rpoC1* transcript encoding the β' subunit of the plastid-encoded RNA polymerase (PEP). Proteomics data provided evidence for the co-existence of both forms (edited/ unedited) of the β' subunit of PEP in Arabidopsis chloroplasts. Poisoned primer extension assays revealed that the ratio between the edited and unedited transcript is highly variable throughout leaf development. Gene expression analysis showed a tight correlation between the level of *FLV* transcript and accumulation of the edited *rpoC1* transcript. Comparison of the plastid transcriptome in white and green leaf sectors of *flv* showed a PEP-deficient profile and a wild-type pattern respectively, indicating a function for RNA editing in the regulation of the PEP activity in chloroplasts.

POS-WED-132

CHARACTERISATION OF TRANSPORT PROTEINS ON THE SYMBIOSOME MEMBRANE OF SOYBEAN (GLYCINE MAX)

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Legumes are an important crop as they provide excellent source of proteins in human diets. Legumes also play an essential role in sustainable agriculture as they are able to form a symbiosis with bacteria (rhizobia) that can fix inert atmospheric nitrogen and make it available to the plant. The plants provide reduced carbon to the bacteria to fuel nitrogenase activity, in exchange for fixed nitrogen. The relationship results in improved soil fertility and also subsequent environmental pollution such as eutrophication of marine systems due to nitrogen run-off. During the legume-rhizobia symbiosis, a new plant organ called the root nodule is induced and developed. Within the root nodule, rhizobia are enclosed in the symbiosome. The symbiosome is central to the interaction; it is an organelle where rhizobia fix atmospheric nitrogen and is surrounded by the plant-derived symbiosome membrane (SM). The SM regulates nutrients exchange between the symbionts, and thus effectively controls the symbiosis. It is therefore critical, that we understand the properties of the SM and identify the transport proteins embedded in it. My aim is to characterize transport proteins within the SM, focusing on three ATP-binding cassette (ABC) transporter proteins that have been identified by proteomic analysis on the SM of soybean. Secondly, I will investigate the involvement of peptidases identified on the membrane in the localisation of mature transport proteins to the SM. Understanding legume-rhizobia symbiosis may lead to future enhancement of legume growth and agricultural productivity, and will reduce nitrate pollution and land degradation.

POS-TUE-133

FUNCTIONAL ANALYSIS OF RIBOSOME ASSEMBLY COFACTORS RELATED TO 30S RIBOSOME SUBUNITS IN CHLOROPLAST

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The ribosome consists of ribosomal proteins and ribosomal RNAs, and it is known that ribosome assembly cofactors are involved in the formation of ribosome. It is reported that the proteins RbfA, Era, RimM and KsgA are involved in the assembly of 30S ribosome in *Escherichia coli*. RimM binds to small subunit protein S19, while RbfA, Era and KsgA are reported to be involved in16S rRNA. Chloroplasts have prokaryotic ribosomes. We discovered a nuclear-encoded chloroplast protein -APG4- whose sequence is homologous to E.coli RbfA. *apg4* mutant was found to have a phenotype with white cotyledons and pale green true leaves. We found that Era, RimM and KsgA all have homologous proteins in *Arabidopsis* - AtEra, AtRimM and Ksg1, which we later discovered had already been identified as PFC1. They showed no phenotype under normal growth conditions. It is reported that the leaves of *pfc1* exhibited chlorosis when grown under cold conditions. When we observed the phenotypes of the nuclear-encoded chloroplast 30S ribosome assembly cofactor mutants under cold conditions, we found that *apg4* and atera leaves showed chlorosis, but atrimM did not. These results suggest that APG4 and AtEra may be important for plastids to grow under cold conditions. When we observed chloroplast rRNA in *apg4*, *atera*, *atrimM* and *pfc1* using RNA blot analysis, we found the accumulation of 16S rRNA in all of the mutants was lower than the same in wild type, and that 23S rRNA and 4.5S rRNA were not efficiently processed in all mutants. These results showed that their proteins are related to the formation of ribosome similar to that in *E. coli*.

POS-WED-134

THE SPECIFIC ROLE OF MITOCHONDRIAL PROTEIN IMPORT FOR GERMINATION

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One unique characteristic of the plant life cycle is that they go through a dormant phase, represented as a seed, lasting from hours to decades. Upon germination, the food reserves in the seed are used to support the plant until autotrophic growth is established. Thus germination and early seedling establishment represent the most crucial stage in the life cycle of plants. Comprehensive transcriptome profiling reveals that mitochondrial establishment represents the one of the earliest molecular events in germinating seeds. Transcriptomic and proteomic profiling throughout germination has uncovered some of the molecular mechanisms involved in mitochondrial biogenesis and has highlighted the importance of the mitochondrial protein import machinery. Tim17-1 encoded by At1g20350, belongs to a large family of proteins termed the Preprotein and Amino acid Tansporters (PRAT), involved in the import of the majority of mitochondrial proteins across the inner membrane. Functional characterisation of T-DNA insertional knock-out lines have revealed that Tim17-1 plays a specific role in germination and may also play a role in plant stress. These results suggest a novel and direct mechanism for the regulation of mitochondrial biogenesis throughout germination.

POS-TUE-135

REGULATION OF *A. THALIANA* MESOPHYLL SV CHANNELS BY PHOSPHOINOSITIDES

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The tonoplast plays an important role in controlling the ionic environment of the vacuole. Accumulation of ions in the vacuole and their subsequent release are fundamental processes for the control of metabolism and osmoregulation in plant cells. Molecular mechanisms mediating tonoplast transport include H^+-pumps (V-ATPase and V-PPase), H^+/ion and H^+/metabolite transporters and ion channels. One of the most abundant tonoplast channels is the SV channel, which has been identified in most, if not all, plant species. In spite of their ubiquity, a clear physiological role for the SV channels is not yet clear, as evinced by the almost normal phenotype of the attpc1 loss-of-function mutant. In order to ascertain the role of the SV tonoplast channels we have initiated a study focused on identifying possible cytoplasmic factors that may regulate their activity. Among possible regulatory molecules, phosphoinositides are well established as signalling elements that regulate membrane trafficking, enzyme and channel activities. Initial results have demonstrated the down regulation of SV channel activity by specific phosphoinositides, with other derivatives having no effect. These and additional results on the effects of phosphoinositides on channel activity and membrane properties will be discussed. This work was supported by Grants IN203112 and IG100513 from DGAPA-UNAM, Mexico.

POS-WED-136

FAX1, A NOVEL MEMBRANE PROTEIN IN THE CHLOROPLAST INNER ENVELOPE INVOLVED IN EXPORT OF FATTY ACIDS AND/OR DERIVATIVES

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Plastids harbor many vital biosynthetic functions during growth and development. Thus, plastid localized metabolite synthesis (e.g. amino acids, fatty acids or secondary compounds) requires extensive solute exchange across the outer and inner envelope membranes. Because fatty acid synthesis in plants exclusively takes place in plastids, export for further lipid metabolism is required. However, until now, knowledge on proteins involved in plastid export of fatty acids and/or derivatives is still scarce. By means of in silico proteomic analysis of plastid envelope proteins, we selected FAX1 (fatty acid export 1), a novel membrane-spanning protein in Arabidopsis thaliana. According to GFP-targeting and immunoblot analysis, FAX1 localizes to the plastid inner envelope membrane. FAX1 knockout mutants (T-DNA insertion) show reduced biomass, thin inflorescence stems and strongly impaired male fertility. In contrast, FAX1 overexpression leads to increased biomass and thicker stems. Transcriptomic, metabolic and ultrastructural analysis imply that FAX1 is a key protein for synthesis of secondary metabolites, such as epidermal waxes or pollen exine layers, which both require fatty acid export from plastids. Further, leaves of FAX1 k.o. show reduced phosphatidylcholine contents, whereas in flowers some triacylglycerols are reduced as well. Reconstitution of FAX1 into yeast mutants supports its fatty acids transport capacity. Based on these observations, we propose a function of FAX1 in export of fatty acids and/or derivatives from plastids.

POS-TUE-137

MULTIPLICITY OF THE EXO70 EXOCYST SUBUNIT IN ARABIDOPSIS

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Before the fusion of secretory vesicles to the plasma membrane at the end of exocytosis, vesicle targeting and tethering is co-regulated by the exocyst complex in eukaryotic cells. The exocyst is highly conserved across eukaryotes and consists of eight subunits (SEC3, SEC5, SEC6, SEC8, SEC10, SEC15, EXO70, and EXO84). In Angiosperms, many subunits are encoded by more than one gene in contrast to yeast and animals. Especially, the *EXO70* gene extremely proliferated into a large gene family – e.g. 23 genes in Arabidopsis. Some EXO70 isoforms show tissue specificity, while other are common, with EXO70A1 being the predominant in Arabidopsis sporophyte (Synek et al., Plant J., 2006). In the same cell, EXO70 isoforms may exhibit redundant, partially overlapping or strictly specific functions. Our aim is to assign specific functions to each subgroup of the *EXO70* family or to each particular isoform. We have inspected insertional mutants in most of *EXO70* genes so far and localized some of them. Novel phenotypic analysis revealed EXO70 isoforms engaged in cytokinesis and pollen tube growth. The study of EXO70 isoforms opens an interesting question, whether each isoform can be incorporated into the exocyst complex or they gained some exocyst-independent functions in the evolution of land plants.

POS-WED-138

EXPO FUNCTION IN PLANTS

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Using the *Arabidopsis* Exo70E2 (AtExo70E2) and its GFP fusion as a probe, we have recently identified a novel double-membrane organelle termed EXPO (exocyst-positive organelle) that mediates an unconventional cytosol to cell wall secretion in plant cells (*Plant Cell* 2010 22: 4009-4030; *Trends Plant Sci* 2012 17: 606-615). EXPO seems to fuse with the plasma membrane (PM) and release a single membrane vesicle into the cell apoplast for exocytosis of cytosolic contents. To further understand the physiological function of EXPO in plants, we first test if EXPO involved in plant defense against pathogens via inoculating transgenic AtExo70E2-GFP *Arabidopsis* or tobacco BY-2 cells with different pathogen stains, followed by confocal observation of possible induced EXPO-PM fusion. To identify EXPO-containing proteins, we also test and use both subcellular fractionation and immuno-isolation approaches to purify EXPO for proteomic analysis and subsequent protein identification. These studies will contribute to our understanding about EXPO function in plants. Here we will present an update on the research progress of these studies. Supported by grants from the Research Grants Council of Hong Kong (GRF & CRF) and CUHK Schemes.

POS-TUE-139

BIOGENESIS OF EXPO IN PLANT CELLS

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The Chinese University of Hong Kong.

Using the *Arabidopsis* Exo70E2 (AtExo70E2) as a probe, we have recently identified an unconventional secretion pathway that is mediated by a novel double membrane structure termed EXPO (exocyst-positive organelle) in plant cells (*Plant Cell* 2010, 22: 4009-4030, *Trends Plant Sci* 2012, 17: 606-615). Our current studies first focus on developing and testing an in vitro budding assay system for studying EXPO formation. Using the Arabidopsis protoplast transient expression system expressing various truncation of AtExo70E2, we next want to map the domains of AtExo70E2 that are essential for its correct subcellular localization and recruitment to EXPO. Preliminary results indicated that multiple domains of AtExo70E2 are involved in its EXPO targeting. Biochemical studies will be further carried out to define the lipid and membrane nature of EXPO-AtExo70E2 interaction. Here we will present an update about our studies in EXPO. Supported by grants from the Research Grants Council of Hong Kong (GRF & CRF) and CUHK Schemes.

POS-WED-140

ACQUISITION, CONSERVATION, AND LOSS OF DUAL-TARGETED PROTEINS IN LAND PLANTS

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The dual-targeting ability of a variety of proteins from *Physcomitrella patens*, rice (*Oryza sativa*), and Arabidopsis (*Arabidopsis thaliana*) was tested to determine when dual targeting arose and to what extent it was conserved in land plants. Overall, the targeting ability of over 80 different proteins from rice and *P. patens*, representing 42 dual-targeted proteins in Arabidopsis, was tested. We found that dual targeting arose early in land plant evolution, as it was evident in many cases with *P. patens* proteins that were conserved in rice and Arabidopsis. Furthermore, we found that the acquisition of dual-targeting ability is still occurring, evident in *P. patens* as well as rice and Arabidopsis. The loss of dual-targeting ability is rare, but does occur, ascorbate peroxidase represents such an example. After gene duplication in rice, individual genes encode proteins that are targeted to a single organelle. Although we found that dual targeting was generally conserved, the ability to detect dual-targeted proteins differed depending on the cell types used. Furthermore, it appears that small changes in the targeting signal can result in a loss (or gain) of dual-targeting ability. The acquisition of dual-targeting ability also appears to be coordinated between proteins. Mitochondrial intermembrane space import and assembly protein40, a protein involved in oxidative folding in mitochondria and peroxisomes, provides an example where acquisition of dual targeting is accompanied by the dual targeting of substrate proteins. Dual targeting of type II dehydrogenates were observed to have evolved in plant evolution and be widespread throughout different plant species.

POS-TUE-141

THE LAST OF THE EDITORS

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It has been over 20 years since the discovery of RNA editing in plants and we are getting closer to a complete understanding of the mechanisms by which this process occurs. Editing is an essential mechanism which modifies target cytidine to uridine bases in both mitochondrial and plastidial messenger RNA. There are 34 known editing sites in *Arabidopsis thaliana* chloroplasts and PentatricoPeptide Repeat (PPR) proteins are strongly implicated in the editing process. There are currently 16 PPR proteins known to account for 21/34 chloroplast editing sites. Using this data, we have found that particular residues in the PPR proteins are involved in target recognition. These residues were used to bioinformatically establish a code which allowed us to align known PPR proteins with their targets. The resulting data strongly suggests that all editing proteins bind 4 nt upstream of the edited C and allowed us to predict the binding factors for unknown editing sites. These predictions have lead to the identification and verification of 3 previously unknown editing factors: AEF1, AEF2 and AEF3 (plAstid Editing Factors) which edit *atpF(12707), ndhB-1(97016)* and *psbE(64109)* respectively. With this technology, we aim to assign editing factors for the remaining sites.

POS-WED-142

FUNCTIONAL CHARACTERIZATION OF MITOCHONDRIAL MECHANOSENSITIVE CHANNEL MSL1 IN *ARABIDOPSIS THALIANA*

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Osmoregulation is the active regulation of the osmotic pressure of cells to maintain the homeostasis of the internal pressure of cells. Especially in plant cells, osmoregulation is involved in a wide range of cellular processes, including cell elongation, stomatal movement and drought tolerance. Mechanosensitive (MS) channels are proteins found in prokaryotic and eukaryotic cell membranes that open in response to mechanical stress. MS channels have been implicated in the cellular osmoregulation as sensor of osmotic pressure. Plants have two types of MS channels; Ca^{2+}-permeable MS channels in plasma membrane, MCA1 and MCA2; and plant homologs of the bacterial MS channels (MscS). Ten MscS like, MSL genes have been identified in Arabidopsis. MSL4-10 genes are targeted to the plasma and vacuolar membranes, whereas MSL2 and MSL3 proteins are localized predominantly to chloroplast envelope and are required for osmoregulation of plastids and normal plastid size/shape. However, mitochondrial MS channels remain to be identified. In this study, we identified MSL1 as the MS channel localized in mitochondria. We will report the role of MSL1 in the osmoregulation of mitochondria.

POS-TUE-143

FROM ROOT HAIRS TO SPINAL NEURONS: TUBULAR ER NETWORK AND DIRECTIONAL CELL GROWTH

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The endoplasmic reticulum (ER) is a highly conserved cellular organelle with interconnected tubules and cisternae stretching throughout the cytoplasm. In polarized growing cells such as root hairs, the ER is constantly formed and reshaped to support rapid cell growth. However, the mechanisms underlying the ER formation and reshaping are not well understood. We have previously showed that Arabidopsis RHD3 (ROOT HAIR DEFECTIVE3), originally identified in a genetic screen for mutants defective in root hair tip growth, plays an essential role in the generation of interconnected ER tubules (Chen et al., 2011). RHD3 is a plant member of dynamin-like atlastin GTPases. Interestingly, improper alterations in Atlsatin-1 in humans cause a medical condition called Hereditary spastic paraplegia (HSP), in which the growth of motor neuron cells from the spinal cord is affected. Atlastin-1 has also been recently shown to be primarily involved in the generation of the tubular ER network in humans. Thus HSP caused by improper alterations in Altastin-1 shares a common underlying cellular defect with abnormal root hair development in *rhd3*. To further understand how atlastin GTPases including RHD3 work inside the polarized growing cells, we conducted a genetic screen for *rhd3-1* modifiers and cloned a gene we call *REN9* (***r**hd3* ***en**hncer9*). *REN9* encodes a microtubule associated protein and it acts together with *RHD3* in the formation of a fine ER network in the tip region of growing root hairs. We will discuss in detail how RHD3 and REN9 act together to control the formation of the ER and directional cell growth. Chen et al. (2011). J. Cell Sci. 124, 2241-2252.

POS-WED-144

AUTOPHAGY AND AUTOPHAGOSOME IN PLANTS

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Autophagy is a conserved catabolic mechanism for cellular protein and organelle control by engulfing them into a structure termed autophagosome. Autophagosome formation requries a serial of steps including the initiation of phagophore/PAS, expantion of the autophagosome membrane, matuation of autophagosome by fusing with the lysosome/endosome and completion of the double membrane autophagosome. However, relatively little is known about the pathways of autophagy and formation of autophagosome in plant cells. In this study, we have used a BAR domain-containing protein as a probe to study the autophagy pathway, the dynamics, formation and membrane origin of autophagosome in plant cells. Using a combination of immunocytochemical (antibodies), molecular (GFP fusions) and genetic (RNAi knockdown) approaches, we show that autophagosome membranes are likely derived from the endoplasmic reticulum (ER) and that this protein plays essential role in the autophagy pathway in *Arabidopsis thaliana*. Here we will present an update on this study. Supported by grants from the Research Grants Council of Hong Kong (GRF & CRF) and CUHK Schemes.

POS-TUE-145

CHARACTERISATION OF A PECTIN METHYLESTERASE INHIBITOR INVOLVED IN CELL WALL DEVELOPMENT AND MUCILAGE STRUCTURE IN THE ARABIDOPSIS SEED COAT

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AtPMEI561 (At1g56100) encodes a putative pectin methylesterase inhibitor protein, identified using microarray analysis. The spatial and temporal expression of *PMEI561* was studied using a (694 nucleotide) promoter:*GUS* construct and qRT-PCR. *GUS* expression was detected in the seed coat, embryo and endosperm of developing seeds at 4 DPA and 7 DPA. At 10 DPA, no *GUS* expression was detected in seeds. The *PMEI561* promoter is also active in leaf tips, petioles, and developing root tissue. T-DNA insertion mutations in *PMEI561* result in mucilage release similar to wild-type seeds when imbibed with water or 50mM EDTA. Scanning electron micrographs of the seed coat epidermis show reduced radial cell wall thickness and increased epidermal cell area. Homogalacturonan (HG) pectin methylesterification in *pmei561* mutant seeds was analysed using an immunofluorescence assay. Mutant mucilage showed wild-type binding of LM19 monoclonal antibody (un-esterified HG) but a severe reduction in LM20 antibody binding (highly methylesterified HG) within mucilage and radial cell walls. Reduced highly methylesterified HG indicates increased PME enzymatic activity in *pmei561* mutant seeds, consistent with mutating a PME inhibitor. Microarray and qRT-PCR analysis suggests that *PMEI561* is regulated by a MYB5/TTG1/bHLH protein complex and by TTG2, independently of GLABRA2. These studies demonstrate that *PMEI561* expression is required for normal seed coat cell wall development and mucilage structure. *PMEI561* may be involved in HG pectin modifications by binding to and regulating the enzymatic activity of at least one PME in developing seeds. The Arabidopsis seed coat is proving a useful model for identifying and characterising genes involved in pectin synthesis and modifications in higher plants.

POS-WED-146

CHARACTERIZATION OF UNDERGROUND STOLON "RHIZOME" OF *CARDAMINE LEUCANTHA* [BRASSICACEAE] BY TRANSCRIPTOME ANALYSIS

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A plant generally grows toward above and below directions. The aboveground part consists of leaves, stem and flower and the belowground part includes rhizome and root. A shoot apical meristem also undergoes two phases, vegetative and reproductive, that are characterized by patterns of growth and organogenesis. Rhizome is an underground shoot elongating horizontally, but lately it develops into an aboveground shoot. Anatomical observation confirmed that a rhizome tip originated from a shoot apical meristem, while a rhizome elongates underground like root. This study aimed to characterize "rhizome" by comparing tramscriptomes of shoot, root and rhizome meristems. RNA was extracted from shoot, rhizome and root apex and leaf in a clonal plant *Cardamine leucantha* [Brassicaceae]. Transcriptome analysis was performed by the paired-end RNA-seq method of HiSeq 2000. Reference sequences were also constructed from cDNA sequences of shoot, leaf, rhizome and root. PCA analysis showed that transcriptome of rhizome was intermediate between those of shoot and root. Especially, expression of genes relating to ER body was high in the rhizome. Therefore, although a rhizome is formed from a shoot apical meristem, gene expression of the organ also corresponds with soil environment where a rhizome elongates.

POS-TUE-147

IDENTIFICATION OF TWO MYB-RELATED TRANSCRIPTION FACTORS, AT4G09450 AND AT2G38090, AS PUTATIVE REGULATORS OF WALL INGROWTH DEPOSITION IN PHLOEM PARENCHYMA TRANSFER CELLS

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Transfer cells (TCs) are anatomically-specialised cells formed at apoplasmic-symplasmic bottlenecks in nutrient transport pathways in plants. TCs are characterized by invaginated wall ingrowths which serve to amplify plasma membrane surface area and thus increase local densities of nutrient transporters required to achieve enhanced flow of nutrients across these bottlenecks. Despite their importance to nutrient transport in plants, little is known of the genetic regulation of wall ingrowth formation. Wall ingrowth deposition in phloem parenchyma (PP) TCs of leaf veins is enhanced in response to high-light, cold and exposure to methyl jasmonate. Exploiting these observations, we used bioinformatics to identify, amongst other genes, 12 MYB-related transcription factors which are up-regulated in leaf tissue in response to these stresses. Phenotypic screening of relevant T-DNA insertional mutants using aniline blue staining of leaf tissue to detect callose associated with the wall ingrowths, identified At4g09450 and At2g38090 as showing significant reductions in the extent of PP TCs in leaves. Phylogenetic analysis showed that these two genes are grouped into separate clades of the MYB-related family, and in silico expression analysis using eFP and Genevestigator showed that both genes are very lowly expressed in leaf tissue, an observation confirmed by qPCR. Based on these characteristics, we are using over expression of At4g09450 and At2g38090 under control of either constitutive (CaMV-35S) or PP-specific (AtSWEET11) promoters, to test whether these genes can function as master regulators of wall ingrowth deposition. Experiments investigating the effect of PP TC knockdown on sucrose export from leaves will also be reported.

POS-WED-148

METABOLIC OSCILLATORS: LINKING NAD+ AND THE CIRCADIAN CLOCK

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The circadian clock is an endogenous mechanism which allows organisms to predict and respond to daily fluctuations in their environment. Over recent years, the structure of the clock has been shown to involve transcription-translation feedback loops, post-translational and post-transcriptional modifications, and most recently biochemical components. In *Arabidopsis thaliana*, cytosolic free calcium (Ca^{2+}_{cyt}) is involved in a feedback loop within the clock, mediated by the calcium-releasing agent cyclic adenosine diphosphate ribose (cADPR). cADPR is synthesised from nicotinamide adenine dinucleotide (NAD+), a coenzyme involved in redox reactions, and integral cofactor during respiration and photosynthesis (as NADP). NAD+ is additionally consumed by poly(ADPribose) polymerases (PARPs), sirtuins (NAD+-dependent histone deacetylases) and ADPRcyc, which synthesises cADPR. Nicotinamide is released as a by-product in all these reactions, and regulates them via feedback inhibition. Nicotinamide treatment lengthens the period of clock outputs in all kingdoms of organisms, supporting the global involvement of NAD+ in clock function. Several lines of evidence point to a role for PARPs and sirtuins in the animal circadian clock mechanism, but their role in plants is only poorly understood. A collection of Arabidopsis T-DNA insertion mutants targeting several pathways of NAD+ metabolism is being screened for circadian phenotypes. This work is being combined with a chemical genomics approach looking at the effect of different inhibitors, including nicotinamide, on the different mutations and circadian phenotypes. This work aims to reveal the target of nicotinamide within the circadian clock, and to decipher the possible relationship between NAD+, a metabolic signal, and molecular clock components. We have shown that a T-DNA insertion in the POLY ADP-RIBOSE GLYCOHYDROLASE 1 (PARG1) gene recapitulates the long-period phenotype previously reported for the tej EMS mutation in the PARG1 gene.

POS-TUE-149

TRANSPORTED SIGNALING MOLECULES THAT REGULATE ROOT STEM CELL HOMEOSTASIS

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The continuous growth style of plants is only possible if a stem cell pool is maintained that will replenish and restore the meristem cell pool. The homeostasis of the root stem cell domain is achieved by signaling between the quiescent centre (QC), stem cells (SC) and the surrounding cells that enter differentiation. However, it is still not understood how cells communicate with each other in order to fine tune the balance between proliferation and differentiation. A known signal molecule in root meristem homeostasis is the CLE40 peptide. *CLE40* is expressed from differentiated columella cells (CCs), and restricts columella stem cell (CSC) fate by regulating the expression of the *WUSCHEL RELATED HOMEOBOX 5 (WOX5)* transcription factor. *WOX5* is specifically expressed in the QC and required to maintain stem cell identity. Experimental data point out that a transmembrane receptor complex consisting of ARABIDOPSIS CRINKLY4 (ACR4) and CLAVATA1 (CLV1) is responsible for perceiving the CLE40 signal. Both *acr4* and *clv1* mutant plants show an increased number of CSCs comparable to *cle40* mutant roots. Moreover *acr4* mutant plants are insensitive towards treatment with CLE40 peptide. Recent work showed that ACR4 and CLV1 specifically interact at the sites of plasmodesmata, suggesting that CLV1-ACR4 complexes regulate the movement of signaling molecules necessary for root meristem homeostasis. A candidate signaling molecule is WOX5, which could move from the QC to the CSCs and hence regulating gene expression promoting stem cell maintenance. In line with this, *wox5* mutants show a non-cellautonomous phenotype in the adjacent CSCs.

POS-WED-150

ACTION OF NF-Y TRANSCRIPTION FACTORS IN PLANT STRESS RESPONSES

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Global food security is one of the prominent challenges facing mankind in a world of increasing population and changing climate. Environmental stresses, such as drought, high salinity and pathogen attack, cause significant crop losses worldwide. Consequently, plants have evolved complex and highly regulated stress response mechanisms. Although there are certainly stimuli-specific pathways, many genes appear to be induced by multiple stresses supporting the existence of a common “core stress response” regulatory network. The NF-Y transcription factor (TF) family are likely key regulators in multiple stress responses. NF-Y TFs function as a heterotrimeric complex consisting of NF-YA, NF-YB and NF-YC subunits, which, in Arabidopsis, are encoded by multigene families that could theoretically combine to form 1690 unique TFs. This combinatorial diversity could enable fine-tuning of transcriptional regulation by activating specific groups of stress-responsive genes. This PhD project aims to identify functional NF-Y complexes involved in regulating plant stress responses, and to elucidate their downstream targets and upsteam regulators. Network inference, together with yeast-1 and 2-hybrid assays and microarray analysis of altered expression mutants has enabled the generation of small-scale networks centred around a subset of key regulatory NF-Y subunits. Current work is focussed on the determination of a complete Arabidopsis NF-Y TF complex, with the recent identification of a putative trimer that is now being investigated *in vivo*.

POS-TUE-151

THE LRR ECTODOMAIN OF THE RECEPTOR KINASE FLS2 NOT ONLY SENSES FLAGELLIN BUT ALSO CONTRIBUTES TO RECEPTOR ACTIVATION

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One important component of plant immunity is the receptor FLS2 (FLAGELLIN SENSING2), which confers resistance against potential pathogens by recognizing the flg22 peptidic epitope of bacterial flagellin. FLS2 is a transmembrane receptor kinase with a large ectodomain composed of 28 leucine-rich repeats (LRRs). FLS2 perceives flg22 by direct interaction in the apoplast and almost immediately after, oligomerises at the plasma membrane with another receptor like-kinase called BAK1 (BRI1-associated kinase1), which positively regulates FLS2 signaling. Within a few seconds, phosphorylation events are observed in the FLS2-BAK1 complex, which likely allow initiation of signaling in the cytoplasm. Although lacking crystal structure data, we recently showed that perception of flg22 by FLS2 relies on two LRR regions of its ectodomain. For this we developed a chimeric receptor approach by swapping LRRs between the tomato and Arabidopsis FLS2 sequences. Among the collection of chimeric receptors generated, we found one specific receptor, which showed an apparent signaling activity in absence of flg22. Here we report on the functional characterization of this chimeric receptor with biochemical, physiological and molecular tools. We demonstrate that its constitutive activity requires exclusively the presence of functional BAK1. Our finding hints at an important function of the ectodomain of FLS2, not only in the perception of its ligand but also, as a molecular platform which decides for the activation of the receptor complex. We propose that the ectodomain present in the chimeric FLS2 allows its specific interaction with BAK1, in a constitutive manner, as a mechanistic explanation for our observations.

POS-WED-152

SPATIO-TEMPORAL TRANSCRIPTOMIC RESPONSES TO NITRATE IN ARABIDOPSIS ROOTS

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Genome-wide transcriptional analyses have provided an impressive catalog of N-responsive genes participating in a wide range of processes. Despite this solid groundwork, the molecular mechanisms involved in regulating and coordinating N-responses at the organism, organ or cellular level are largely unknown. In addition, the majority of these genome-wide studies were performed at defined time points in whole plants or organs impairing our understanding of cell-specific regulatory gene networks and how they interact to coordinate organ responses over time. In this research, we map and characterize dynamic N-regulatory networks acting within and/or between cell types in Arabidopsis roots. Our goal is to understand how cell-specific genome-wide responses are orchestrated to produce coherent organ responses over space and time under nitrate treatments. To address this question, we combined cell-sorting, transcriptomics analysis and integrative network bioinformatics to identify cell type specific regulatory gene networks controlling root responses to nitrate over time. We have been able to characterize N-responsive genes with specific regulation patterns at cell-type level over time. This analysis revealed many N-responsive genes specifically modulated in a spatial or temporal manner, with tissue specific gene clusters showing significant enrichment for different gene ontology terms. These findings suggest a fine tune control over gene responses to N at both cell-type and temporal levels. MilenioP10-062-F, Fondap1509007, Fondecyt110698, Proyecto Genoma 07, HHMI, Beca Apoyo Tesis 24121433, ANR-007 and CONICYT doctoral fellowship grants.

POS-TUE-153

THE ARABIDOPSIS CYCLIC NUCLEOTIDE INTERACTOME SUGGESTS A ROLE FOR CYCLIC NUCLEOTIDES IN THE REGULATION OF PHOTORESPIRATION DURING THE DEFENSE RESPONSE

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In plants, the presence of cyclic nucleotides (CNs) has been unequivocally established however the downstream CN mediated signal transduction pathway is not well understood. This is largely because the protein targets of CNs are unknown. Plant genomes lack sequences that have significant homology to the protein kinases, guanine-nucleotide-exchange factors and phosphodiesterases that bind CNs in animals. Currently the main targets of CNs in plants are thought to be a family of cyclic nucleotide gated ion channels. This study aimed to identify cyclic nucleotide binding proteins (CNBPs) in Arabidopsis. Agarose or biotin coupled cyclic AMP and cyclic GMP were used to affinity purify interacting proteins from leaf and callus extracts. Additionally, cyclic AMP or cyclic GMP antibodies were used to immunoaffinity purify leaf and callus proteins bound to endogenous CNs. A total of 13 candidate proteins were identified that were consistently purified with the different CN baits and many of these contain sequences that resemble known CN binding domains. A transcriptional analysis revealed that nine of the candidate CNBPs are highly coexpressed and encode key enzymes involved in the supply of carbon dioxide to Rubisco, the Calvin cycle and photorespiration, including the production of hydrogen peroxide for the hypersensitive response against pathogens. Interestingly, most of the CNBP candidates have been shown to be modified by nitric oxide. We therefore propose that these candidate CNBPs are points of cross talk for multiple signal transduction pathways and regulate the flux through the photorespiratory pathway during the defense response.

POS-WED-154

THE INFLUENCE OF AUXIN TRANSPORT INHIBITORS ON CHLOROPLAST MOVEMENT AND PHOTOTROPIN EXPRESSION IN *ARABIDOPSIS THALIANA*

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The role of auxin in plants and the functioning of the plant cytoskeleton have both been widely studied for many years. Recently, more and more links between the cytoskeleton and auxin are emerging. For example, both of these components take part in establishing and maintaining cell polarity. Auxin is also known to play a major role in phototropism, a process controlled by blue light via phototropins. On the other hand, both phototropins and the actin cytoskeleton are key elements in the process of chloroplast movement. These connections between auxin and blue light signaling pathways suggest the existence of a link between auxin and chloroplast movements. In this study auxin transport inhibitors were used to investigate the role of auxin in blue light-induced chloroplast relocations. *Arabidopsis thaliana* plants were cultured *in vitro* on inhibitor-containing media. Several different auxin influx and efflux inhibitors were tested. In mature plants the expression of phototropin 1 and 2 was evaluated at the mRNA level and chloroplast responses were measured using a photometric method. Chloroplast movement parameters and phototropin expression levels were reduced in some of the inhibitor-treated plants. These differences point to a modulatory role of auxin in the signaling pathway controlling chloroplast relocations.

POS-TUE-155

AMBIENT TEMPERATURE RESPONSE AND SERENE/ARGININE PROTEIN MEDIATED ALTERNATIVE SPLICING IN *ARABIDOPSIS THALIANA*

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Alternative splicing is a well-documented mechanism for posttranscriptional gene regulation in higher eukaryotes. Alternative splicing is prevalent in plants: relatively few studies however provide functional evidence for alternative splicing and the regulation of key plants processes. Temperature is a key environmental signal that plants continually monitor and assimilate. Plants respond to high temperature conditions with altered growth and development; for example, higher temperatures accelerates flowering. Little is known, however, of the underlying genetic basis for temperature perception and response. Here we use natural variation and reverse genetics to describe a role for serine/arginine (SR) protein splice factors in temperature response in Arabidopsis. We have identified SR protein genes that show altered transcription profiles when grown at higher ambient temperatures (28° C, versus 23° C), and that are differentially regulated in natural accessions that vary in response to higher temperatures. We describe two mutants that exhibit phenotypes relating to disruption in thermal response that is reminiscent of *pif4* mutants: for example, reduced hypocotyl length, rosette size and flowering time defects are observed with corresponding molecular phenotypes. SR protein gene transcripts themselves undergo extensive alternative splicing. We are currently mapping natural variation in the splicing patterns of one of the SR proteins modulated by temperature. Our studies suggest a role for SR protein splice factors in thermal responses of Arabidopsis thaliana.

POS-WED-156

TRANSCRIPTOME ANALYSIS OF DESICCATION TOLERANCE IN SEEDS OF *ARABIDOPSIS THALIANA*

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Desiccation tolerance (DT) has allowed seed plants to conquer ecosystems with long periods of limited water availability. This adaptive features allows seeds to remain dried for very long times without losing their ability to germinate. Several components of the DT process have been proposed including LEA and heat shock proteins and the accumulation of oligosaccharides and other osmoprotectants. In Arabidopsis several mutants lacking DT have been reported, such as *lec1* mutant (leafy cotyledon 1), *fus3* (fusca 3) and *abi3* (abscisic acid-insensitive 3), which lack or have reduced some of the proposed DT components. However, there is little information about all the components required to achieve DT and on how these transcription factors modulate the global DT process. We performed RNA-seq experiment and carbohydrates profiles of *lec1*, *lec2*, *fus3* and *abi3*, as well as their wild type at three stages of seed development 15, 17 and 21 DAF (day after open flower) belonging to the seed desiccation period. A complex experimental design approach and regulatory networks prediction were used to identify differentially expressed geneΔs involved specifically in DT process. Our findings show that transcripts affected in intolerant mutants seeds are repressed including those encoding LEA, seed storage and abiotic stress related proteins as well as decreasing in amount of oligosaccharides such as raffinose and stachyose. On other hand, there are a massive induction of genes belonging to many other classes such as photosynthesis, cell wall modification and energy metabolism, probably related to the direct pass from embryo to vegetative development. These data enabled connection of metabolic processes, signaling pathways, and specify transcription factor TF activity. Using network models we identified two major transcriptional networks related to storage of reserve compounds and cellular protection mechanisms, respectively. We are currently testing the DT of mutants affected in the major node genes of these networks and whether some TF downstream of LEC1, ABI3 and FUS3 play a key role in regulating the desiccation tolerance process.

POS-TUE-157

NITRATE REGULATORY MECHANISMS INVOLVED IN THE CONTROL OF FLOWERING TIME

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Nitrogen (N) is an essential macronutrient and its availability is one of the primary factors limiting plant growth and agricultural productivity. N nutrient/metabolites can have profound impact on root development, flowering time and other developmental programs. Some of the regulatory gene networks mediating root developmental responses to N availability have been identified. However, despite the economic importance of understanding the relationship between plant N nutrition and flowering, relatively little is known about the molecular mechanisms that control flowering time in response to N supply. In this study, we used systems approaches to identify regulatory factors involved in N-control of flowering time in Arabidopsis. To analyze the possible role of these genes in the N-response, we evaluated the effect of nitrate treatments on their expression. We found that these genes accumulated quickly after nitrate treatments. Arabidopsis mutant lines verified the importance of these genes for control of flowering time in response to N. Our work defined a model for N control of flowering time in Arabidopsis.

POS-WED-158

ELF3 AS A REPRESSIVE HUB: ALTERNATIVE ELF3 REPRESSIVE COMPLEXES ACROSS TEMPERATURE

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The circadian clock is an endogenous time-keeping mechanism that allows plants to respond in the most beneficial way to the external environment. The clock is entrained by inputs into the system including light and temperature. It is important that the clock is capable of maintaining period length over a range of ambient temperatures, this is known as temperature compensation. The ROBuST (Regulation of Biological Signalling by Temperature) project has studied the mechanisms behind temperature compensation in Arabidopsis. One example of a temperature specific response in controlling clock genes is the Evening Complex. The Evening Complex consists of three proteins, the LUX binding protein, ELF3 adaptor protein and ELF4 nuclear localisation protein. Together these proteins repress the expression of target genes (*PRR9*, *PIF4* and *PIF5*) by binding to the LUX binding site in the promoter regions of targets. We show that whilst the traditional evening complex (LUX-ELF3-ELF4) is sufficient to repress target genes in the warm it is insufficient in the cool. At cooler temperatures only ELF3 is required. ELF3 is not capable of binding to promoters so we explored the hypothesis that ELF3 switches between repressive complexes under a range of ambient temperatures. By switching between complexes we show that the repressive role of ELF3 is not exclusively through the DNA binding protein LUX.

POS-TUE-159

INTERACTION OF NITROGEN FORMS AND AUXIN TO PROMOTE ROOT FORMATION AS REVEALED BY THE ANALYSIS OF AN ARABIDOPSIS AMMONIUM TRANSPORTER MUTANT

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Nitrogen (N, ammonium, and nitrate forms) is an essential macronutrient for plants, and therefore there is an increasing demand for N in global crop production. Understanding N use efficiency and its dependence on diversity of N forms remains as one of the biggest challenges in agriculture. Auxins are phytohormones involved in plant root development. The action of auxin on controlling root system architecture is known to be affected by nitrate uptake and assimilation. In this study, a quadruple knockout mutant (qko) of Arabidopsis (A. thaliana) ammonium transporters was generated in the background of DR5-GFP auxin reporter line to elucidate the cross-talks between ammonium transport and auxin signaling pathway. We performed a comparative analysis of qko mutant and wild-type Arabidopsis plants that displayed different growth of primary root (PR) and lateral roots (LR) depending on N forms and auxin accumulation. We identified a relationship between PR growth on ammonium and expression of DR5-GFP. The effect of N forms and ammonium transporters on auxin response and root growth phenotypes will be discussed on this poster presentation.

POS-WED-160

FUNCTIONAL PROMOTER ANALYSIS OF GRXC9, A GENE ACTIVATED BY A NON-CANONICAL SALICYLIC ACID-DEPENDENT PATHWAY IN ARABIDOPSIS

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Salicylic acid (SA) is one of the key hormones involved in defense responses against some environmental conditions. *GRXC9* gene, coding for a glutaredoxin with a putative antioxidant function, belongs to a group of genes early and transiently activated by SA. GRXC9 is also activated by different stress conditions by a SA-dependent manner and is partially dependent of the transcriptional co-activator NPR1. We are interested to elucidate the mechanism controlling the activation of this gene by SA. *In silico* analysis of GRXC9 promoter, allow us identified two putative SA-responsive *as-1*-like elements in its proximal region. These elements have been described as targets of transcription factors of the bZIP TGA family. By using different constructs of *GRXC9* promoter fused to GUS, we show that both *as-1*-like elements are needed for *GRXC9* SA-mediated induction. The expression of *GRXC9* is abolished in the triple *tga2/5/6*. Then we analyzed the binding of different class of TGA transcription factors to the GRXC9 promoter by Chromatin Immunoprecipitation assay. Interestingly, we found that in wild type plants, TGA2 and TGA3 are constitutively bound to *GRXC9* promoter,; however only after SA stimuli the RNA polymerase II is recruited to the promoter. This effect is not observed in the *tga2/5/6* triple mutant plants, indicating that the RNA pol II recruitment is dependent of SA and the TGA class II proteins. Our results indicate that under basal conditions TGAs are bound to *GRXC9* promoter and the SA stimuli produces a change in TGA complex allowing the recruitment of RNA pol II and the transcriptional activation of *GRXC9*. Supported by FONDECYT 1100656 and Millennium Nucleus P10-062-F.

POS-TUE-161

HUNTING FOR GENES THAT LINK CIS-CAROTENES TO CHLOROPLAST DEVELOPMENT

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Carotenoids play vital roles in plant development and survival, and carotenoid composition is tuned to the developmental stage, tissue, and to environmental stimuli. After a decade of advances in understanding of biosynthetic enzymes, the next frontier is to understand what regulates carotenoid biosynthesis, accumulation and storage. *Cis*-carotenoids have been hypothesized to mediate chloroplast to nuclear communication by controlling nuclear gene expression. The *carotenoid isomerase* (CRTISO, *ccr2*) is a key rate-limiting step and loss-of-function leads to the accumulation of *cis*-carotenoids, reduces lutein, perturbs chloroplast development in new leaf tissues (leaf yellowing) and alters plant development. A forward genetics screen of EMS-mutagenised *ccr2* seeds identified ~20 revertant *ccr2* plants (*rccr2*) that show normal greening of leaf tissues, yet still have reduced lutein and an over accumulation of *cis*-carotenoids. Next generation sequencing is being used to identify candidate genes underpinning *rccr2* mutant loci and functional characterization of these genes is underway. We anticipate the discovery of a novel *cis*-carotenoid signaling pathway that relays signals from the chloroplast to control plant development and reproduction.

POS-WED-162

DIFFERENTIAL REGULATION OF ARABIDOPSIS PLASTID GENE EXPRESSION AND RNA EDITING IN NON-PHOTOSYNTHETIC TISSUES

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RNA editing is one of the post-transcriptional processes that commonly occur in plant plastids and mitochondria. In Arabidopsis, 34 C-to-U RNA editing events, affecting transcripts of 18 plastid genes, have been identified. Here, we examined the editing and expression of these transcripts in different organs, and in green and non-green seedlings (etiolated, cia5-2, ispF and ispG albino mutants, lincomycin-, and norflurazon-treated). The editing efficiency of Arabidopsis plastid transcripts varies from site to site, and may be specifically regulated in different tissues. Steady state levels of plastid transcripts are low or undetectable in etiolated seedlings, but most sites are edited with efficiencies similar to those observed in green seedlings. By contrast, the editing of some sites is completely lost or significantly reduced in other non-green tissues; for instance, the editing of ndhB-149, ndhB-1255, and ndhD-2 is completely lost in roots and in lincomycin-treated seedlings. The editing of ndhD-2 is also completely lost in albino mutants and norflurazon-treated seedlings. However, matK-640 is completely edited, and accD-794, atpF-92, psbE-214, psbF-77, psbZ-50, and rps14-50 are completely or highly edited in both green and non-green tissues. In addition, the expression of nucleus-encoded RNA polymerase dependent transcripts is specifically induced by lincomycin, and the splicing of ndhB transcripts is significantly reduced in the albino mutants and inhibitor-treated seedlings. Our results indicate that plastid gene expression, and the splicing and editing of plastid transcripts are specifically and differentially regulated in various types of non-green tissues.

POS-TUE-163

IDENTIFICATION OF PROTEIN-PROTEIN INTERACTIONS REGULATING SODIUM-PROTON ANTIPORTER ACTIVITY

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In plants, the concentration and ratio of Na^+ and K^+ is crucial for homeostasis, particularly in stressful conditions such as salinity, cold and drought. Ion homeostasis in plants is regulated by a large number of ion exchangers that control the movement of ions across the plasma and intracellular membranes. The class of sodium-proton exchangers designated as NHEs in animals or NHXs in plants and yeast, is highly conserved throughout eukaryotes. NHX5 and NHX6 are intracellular-localized proteins that are widely conserved in diverse plants. The C-terminal tail of NHX5 and NHX6 is also well conserved in many plant species, suggesting that it may mediate a protein-protein interaction and act as a part of pH sensing mechanism. To investigate the functional significance of this well conserved C-terminal tail in NHX5 and NHX6, a yeast 2-hybrid screen has been performed to identify interacting proteins. The predicted C-terminal tail and the highly conserved regions of both NHX5 and NHX6 have been used as baits to screen existing 2-hybrid libraries. Positive colonies were identified, and several of these interactions have been validated by BiFC (bimolecular fluorescent complementation) assay. These interaction further will be analyzed using by deletion analysis of both the C-terminal region of NXH6, and the interacting protein(s).

POS-WED-164

A UNIQUE APPROACH TO IDENTIFY AND VALIDATE NOVEL REGULATORY PEPTIDE-CODING GENES

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Plants co-ordinate their growth and development under remarkably diverse climatic and environmental conditions but how they do this is not completely understood. Having to process information without brains or nervous system, the plants have evolved complex intercellular regulatory systems including peptide-mediated signalling pathways to regulate growth, development and responses to their environment. However, only about 15 families of regulatory peptide coding genes are known and the function of only a few have been elucidated to some extent. Using a unique approach to specifically screening for small secreted peptide coding genes that have strong conserved domains, we were able to identify not only new members of existing families but novel families for putative regulatory peptide coding genes across many plant species including Arabidopsis. Gene expression analysis in combination with loss-of-function, constitutive expression and biochemical approaches have been used to validate the prediction and determine the regulation and function of some of these genes and our findings reveal roles for regulatory peptides in mediating plant development in response to environmental stimuli.

POS-TUE-165

UNRAVELING THE GENE REGULATORY NETWORK OF A SENESCENCE-ASSOCIATED NAC TRANSCRIPTION FACTOR IN *ARABIDOPSIS THALIANA*

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

POS-WED-166

OVEREXPRESSION OF THE SWEETPOTATO R2R3-TYPE *IBMYB1A* GENE ACTIVATES ANTHOCYANIN PRODUCTION IN HETEROLOGOUS PLANTS

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R2R3-type MYB transcription factors play important roles in transcriptional regulation of anthocyanins. The R2R3-type IbMYB1 is known to be a key regulator of anthocyanin biosynthesis in the storage roots of sweetpotato. We previously showed that transient expression of *IbMYB1a* led to anthocyanin pigmentation in tobacco leaves. In this presentation, we generated transgenic *Arabidopsis*, tobacco, and alfalfa plants expressing the *IbMYB1a* gene under the control of *CaMV 35S* promoter, and the sweetpotato *SPO* and *SWPA2* promoters. Overexpression of *IbMYBa* in heterologous plants produced strong anthocyanin pigmentation in seedlings and generated a deep purple color in leaves, stems, and seeds. RT-PCR analysis showed that *IbMYB1a* expression induced the activation of a subset of genes involved in the anthocyanin biosynthetic pathway. Furthermore, overexpression of *IbMYB1a* in *Arabidopsis* led to enhanced expression of the *AtTT8* (*bHLH*) and *PAP1/AtMYB75* genes. HPLC analysis revealed that *IbMYB1a* expression led to the production of cyanidin as a major core molecule of anthocyanidins in transgenic plants, as occurs in the purple leaves of sweetpotato (cv. Sinzami). Our results demonstrate that the IbMYB1a transcription factor is sufficient to induce anthocyanin production through transcriptional regulation of several structural genes involved in anthocyanin biosynthesis. These results suggest that the single IbMYB1a transcription factor can be exploited for the enhanced production of anthocyanins, which have important nutritive values in plant species of interest.

POS-TUE-167

CHARACTERIZATION OF TRANSCRIPTION FACTOR *HAT2* USING T-DNA MUTANTS

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In Arabidopsis, the embryo-specific transcription factor LEAFY COTYLEDON 2 (LEC2) plays key roles in embryogenesis. To identify genes regulated by LEC2, microarray analysis was performed in leaves of senescence-inducible *LEC2* and wild type (WT) Arabidopsis plants. We found sixteen upregulated and eighteen downregulated genes encoding transcription factors compared with WT. We selected one upregulated *HAT2* gene to further characterize its function. The *HAT2* encoding a homeodomain-leucine zipper protein. RT-RCR analysis was performed to examine the relative abundance of *HAT2* mRNA in different plant organs. *HAT2* is expressed ubiquitously, in seedling and mature plants, leaves, stems, flowers, root and siliques. To detailed analysis of function of HAT2, we obtained two homozygous mutant lines, *hat2-1* and *hat2-2*. We discovered that the *hat2-2* mutant expresses a truncated mRNA. To examine whether HAT2 affects on expression of other genes, expression of thirteen genes were tested by RT-PCR analysis using cDNA from siliques. Three genes (*MPK1*0, *HAT14*, AT5G51910) were upregulated in *hat2-1* mutant but they were downregulated in *hat2-2* mutant plant. This result suggests that HAT2 is a negative regulator of some genes expression, and it supposed that *hat2-2* mutant acts like HAT2 overexpressing plant. In addition, *hat2-2* mutant showed earlier bolting and smaller size phenotype than WT plants.

POS-WED-168

THE CENTRAL ROLE OF NLP TRANSCRIPTION FACTORS IN NITRATE-INDUCIBLE GENE EXPRESSION

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For land plants, nitrate is a major nitrogen source but it is also a signaling molecule that modulates the expression of a wide range of genes and that regulates growth and development. The critical role of nitrate as a signaling molecule has been established for decades. However, the molecular mechanism underlying gene expression in response to nitrate signal has remained elusive, as the transcription factor that mediates the nitrate signal had not yet been identified. To identify such a key transcription factor, we conducted yeast one-hybrid screening using the nitrate-responsive *cis*-element (NRE), which we previously identified in the nitrite reductase gene promoter (1). The results revealed that NIN-LIKE PROTEIN (NLP) family proteins are NRE-binding proteins. The RWP-RK domain in NLPs was found to mediate the binding to the NRE and an NRE-like sequence at the locus for a nitrate reductase gene. We also found that NLP activates NRE-dependent transcription through the region N-terminal to the RWP-RK domain in a nitrate-dependent manner and also that the activity of NLP is likely modulated by nitrate signalling in a posttranslational manner. The suppression of NLP function impaired the nitrate-inducible expression of a number of genes, including genes involved in nitrate assimilation and putative transcription factor genes, and caused severe growth inhibition. These results suggest that NLP transcription factors play a central role in nitrate response as master regulators of the nitrate-inducible gene expression (2). (1) Konishi, M. and Yanagisawa, S. (2010) Plant J., 63, 269-282. (2) Konishi, M. and Yanagisawa, S. (2013) Nat. Commun., in press.

POS-TUE-169

IDENTIFICATION AND CHARACTERISATION OF A NOVEL MITOCHONDRIAL STRESS-RESPONSIVE PROTEIN

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A defining feature of the eukaryotic cell is the presence of distinct membrane bound compartments, known as organelles. Specific organelles accommodate a diverse but defined assembly of enzymes, whose compartmentalisation has allowed for a level of metabolic control unattainable in prokaryotic cells. The transfer of mitochondrial genes to the nucleus has prompted the evolution of a series of mechanisms for regulating and coordinating nuclear and mitochondrial functions. There are two fundamental signalling pathways associated with the regulation of gene expression of organelle proteins. These are commonly referred to as anterograde and retrograde signalling pathways. Induction of the alternative oxidase has long been the gold standard for characterising mitochondrial retrograde signalling. However, AOX induction has been shown to occur in response to chloroplast stress in addition to mitochondrial stress. Consequently, several analyses were carried out to identify additional putative targets to serve as models for mitochondrial retrograde regulation. Analysis of a number of T-DNA insertion mutants in genes encoding mitochondrial proteins identified one gene whose transcript abundance was up-regulated in a number of mutants, from 10 to 60 fold in abundance. Analysis of T-DNA knock-outs of this genes display stress resistant phenotypes. The functional characterisation of this protein will be presented.

POS-WED-170

RAPID VACUOLAR STRUCTURAL CHANGES IN GUARD CELLS REQUIRE PHOSPHATIDYLINOSITOL 3,5-BISPHOSPHATE

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Rapid stomatal closure is essential for water conservation by the plant, which is critical for survival under water deficiency. To close stomata rapidly, guard cells reduce their volume by converting the large central vacuole into a highly convoluted structure. However, the molecular mechanisms underlying this change are poorly understood. In this study, we used pH-indicator dyes to demonstrate that vacuolar convolution is accompanied by acidification of the vacuole in *Vicia faba* guard cells during ABA-induced stomatal closure. Vacuolar acidification is necessary for the rapid stomatal closure induced by ABA, since a double mutant of the vacuolar H+-ATPase *vha-a2 vha-a3* and vacuolar H+-PPase mutant *vhp1* showed delayed stomatal closure. Furthermore, we provide evidence for the critical role of phosphatidylinositol 3,5-bisphosphate (PtdIns(3,5) P2) in the changes in pH and morphology that take place in the vacuole. Single and double *Arabidopsis thaliana* null mutants of phosphatidylinositol 3-phosphate 5-kinases (PI3P5Ks) exhibited slow stomatal closure upon ABA treatment compared to the wild type. Moreover, an inhibitor of PI3P5K reduced vacuolar acidification and convolution, and delayed stomatal closure in response to ABA. Taken together, these results suggest that rapid ABA-induced stomatal closure requires PtdIns(3,5)P2, which is essential for vacuolar acidification and convolution.

POS-TUE-171

PARENTAL IMPRINTING OF *UCL1* REGULATING CURLY LEAF POLYCOMB PROTEIN ACTIVITY IN ARABIDOPSIS

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The Arabidopsis PRC2 complex controls homeotic gene expression, flowering time and gene imprinting. Although downstream target genes and the regulatory mechanism of the PRC2 complex which is conserved in higher eukaryotes are well understood, much less is known about the significance of post-translational regulation of PRC2 protein activity. Here, the post-translational regulation of CLF SET-domain PcG protein by the F-box protein, UPWARD CURLY LEAF1 (UCL1) is present. Overexpression of *UCL1* generates mutant phenotypes similar to those observed in plants with a loss-of-function mutation in the *CLF* gene and the overexpression of *UCL1* reduces the level of CLF protein and alters expression and H3K27 methylation of CLF-target genes in transgenic plants, suggesting that UCL1 negatively regulates CLF in developing endosperm. We also found that *UCL1* is primarily expressed paternally but repressed maternally in the endosperm after fertilization. The silenced *UCL1* maternal allele is derepressed in some PcG mutant endosperms as well as in mutant embryo sacs. Because expression of *UCL1* is altered in the plants pollinated with the hypomethylation mutant, as compared to the plants pollinated with wild type, DNA methylation seems to be important for the paternal expression of *UCL1*. These suggest that the maternal allele of *UCL1* is repressed by the PcG complex and that some conserved or divergent mechanisms for the regulation of paternal imprinting had been adopted during evolution in Arabidopsis. These results demonstrate that *UCL1* regulating post-translationally the CLF SET-domain PcG activity is controlled by parental imprinting in the Arabidopsis endosperm.

POS-WED-172

ARABIDOPSIS DREB2C TRANSCRIPTION FACTOR ACTS AS AN ACTIVATOR OF THE EXPRESSION OF THE HEAT-INDUCIBLE *PHYTOCYSTATIN 4* GENE *ATCYS4*

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Phytocystatins are proteinaceous inhibitors of cysteine proteases. They have been implicated in the regulation of plant protein turnover and in defense against pathogens and insects. Here, we have characterized an *Arabidopsis* phytocystatin family gene, *AtCYS4* (*Arabidopsis thaliana phytocystatin 4*). *AtCYS4* was induced by heat stress. The heat shock (HS) tolerance of *AtCYS4*-overexpressing transgenic plants was greater than that of wild-type and *atcys4* knock-down plants, as measured by fresh weight and root length. Although no heat shock elements were identified in the 5'-flanking region of the *AtCYS4* gene, canonical ABA-responsive elements (ABREs) and dehydration-responsive elements (DREs) were found. Transient promoter activity measurements showed that *AtCYS4* expression was up-regulated in unstressed protoplasts by co-expression of DRE-binding factor 2s (DREB2s), especially by DREB2C, but not by bZIP transcription factors that bind to ABREs (ABFs, ABI5 and AREBs). DREB2C bound to and activated transcription from the two DREs on the *AtCYS4* promoter although some preference was observed for the GCCGAC DRE element over the ACCGAC element. *AtCYS4* transcript and protein levels were elevated in transgenic *DREB2C* overexpression lines with corresponding decline of endogenous cysteine peptidase activity. We propose that *AtCYS4* functions in thermotolerance under the control of the DREB2C cascade.

POS-TUE-173

IN SILICO ANALYSIS OF CELL-TYPE SPECIFIC OMIC RESPONSES REVEAL NOVEL SPATIOTEMPORAL REGULATORY NETWORKS REGULATING PHOSPHATE ACQUISITION DURING PI STRESS IN ARABIDOPSIS AND RICE

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Despite an evolutionary divergence over 150 myr, Arabidopsis and rice share numerous orthologous genes and gene regulatory networks. One of these conserved networks includes the central regulator mediating the systemic signalling response during phosphate starvation. Phosphate (Pi) is an essential nutrient for all organisms, and is required for many key processes in plants. Thus, a better understanding of Pi metabolism would contribute towards the development of plants with higher Pi-acquisition efficiency. Through the coupling of cell-type isolation and transcriptomic profiling, novel insights have been gained regarding the global transcript abundance in each discrete cell-type under control conditions. Here, a bioinformatic analysis was performed to identify putative cell-type specific regulators in Arabidopsis and rice roots. By utilising data from cell-type specific transcriptomes, together with transcriptomes of whole-roots subjected to Pi starvation, cell-type specific Pi-stress responses could be deduced. Similar to other cell-type specific profiling approaches to various nutrient deficiencies, including nitrogen (N) and iron (Fe) limitation [1,2], the majority of certain biological processes were associated with one or two cell-types. With special emphasis on the Pi high-affinity transporters that were enriched in epidermal and vasculature tissue, a subset of genes, which were co-enriched in the same cell-types (and are Pi-responsive, co-expressed and putative protein-protein interactors) were identified in both species. These candidate genes will be experimentally validated in future and functionally characterised for their cell-type specific role during Pi starvation. References: 1. Gifford et al. PNAS USA **2008**, 105, 803-808. 2. Long et al. Plant Cell **2010**, 22, 2219-2236.

POS-WED-174

COMPREHENSIVE ANALYSIS OF THE *ARABIDOPSIS* STIGMATIC PAPILLA CELL TRANSCRIPTOME

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In *Arabidopsis*, pollination occurs on a surface of the stigmatic papilla cells. Pollination system is categorized as pollen recognition, supply of water for pollen and pollen tube guidance to stigmatic surface. To understand these molecular mechanisms, we conducted an accurately and comprehensive transcriptome analysis of *Arabidopsis* papilla cell, by a combination of laser microdissection (LM) and high-throughput sequencing. The papilla cells of *A. thaliana* and *A. halleri* were specifically isolated by LM, and total RNAs were extracted. After sample preparation, transcripts were sequenced using the SOLiD5500xl, and sequence reads were mapped to the *A. thaliana* genome (TAIR10), as a reference genome sequence. Bioinformatics analysis was performed with various databases for their data mining. Consequently, 65 million to 180 million reads were obtained from the papilla cells of both species, approximately 14,000 to 16,000 genes were estimated to be expressed in the papilla cells, and 80% of genes were common between both species. In classification of common genes by gene ontology (GO), GO terms of metabolism, transcription, protein phosphorylation and transport were predominant. These results suggest that a variety of molecular mechanisms such as signaling and nutrient transport for pollen are actively operating in the papilla cell and their coordination is one of the keys for successful pollination.

POS-TUE-175

CHARACTERIZATION OF THE FUNCTION OF CALMODULIN-LIKE (CML)23 AND CML24 IN THE *ARABIDOPSIS THALIANA* CIRCADIAN CLOCK

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CML23 and CML24 are potential Ca^{2+} sensors that are involved in the regulation of the *Arabidopsis thaliana* circadian clock. These plant-specific family of Ca^{2+} sensor proteins may have overlapping roles in regulating the Arabidopsis central circadian oscillator. A previous study proposed that transcription of core oscillator genes are affected in the *cml23*-2*cml24*-4 double mutant. However, how the circadian oscillator is regulated by CML23 and CML24 remains to be explained because CML23 and CML24 are not thought to be transcription factors. We hypothesize that CML23 and CML24 are cytosolic proteins, which play a role in signal transduction from the cytosol into the nucleus. It is likely that CML23 and CML24 regulate the circadian oscillator through interacting intermediaries. Conformational changes undergone by CML23 and CML24, as a consequence of Ca^{2+} binding to the 4 EF hands, may facilitate the recognition of target proteins. Study of the epistatic relationship between core oscillator genes and *cml23*-2*cml24*-4 may indicate potential points of entry for CML23 and CML24 into the circadian system. In this study, co-immunoprecipitation and epistatic analysis are used to characterize the function of CML23 and CML24 in the regulation of the Arabidopsis thaliana circadian clock.

POS-WED-176

GENOMIC ANALYSIS OF IRE1-DEPENDENT DECAY OF mRNAs IN *ARABIDOPSIS THALIANA* REVEALS A CONNECTION BETWEEN UNFOLDED PROTEIN RESPONSE AND SEVERAL PHYSIOLOGICAL PLANT PROCESS

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The unfolded protein response (UPR) is a cellular process triggered by the accumulation of misfolded protein in the lumen of endoplasmic reticulum. IRE1 is a transmembrane protein involved in the sensing and signaling during the UPR. During this process in *Arabidopsis thaliana*, IRE1 cleaves the AtbZIP60 mRNA leading to the formation of a processed form of AtbZIP60 that regulates the expression of UPR-related genes. In other eukaryotes, it has been reported that IRE1 can cleavage other mRNAs leading to a phenomenon known as Regulated IRE1 Dependent Decay (RIDD). To evaluate whether RIDD is activated during UPR in *Arabidopsis thaliana*, we performed a transcriptomic analysis of wild type plants treated with two UPR-inducer compounds (DTT or Tunicamycin (Tm)). Under these conditions, several genes were down regulated. To determine what genes were down regulated due to IRE1, we performed the same analysis in an IRE1 double mutant plant (Arabidopsis has two IRE1 genes, IRE1a and IRE1b). Many of the genes down regulated in the wild type were not altered in the *ire1a ire1b* mutant subjected to DTT or Tm treatments, suggesting that these genes are targets of IRE1. The analysis of this set of genes show that they belong to gene families involved in cell wall, lipids and hormone metabolism. The decay of some of these transcripts was confirmed by RT-qPCR analyses using IRE1 single and double mutant plants treated with tunicamicyn or DTT. Our data led us to propose that regulated IRE1-dependent decay of mRNAs could be involved in plant processes such as plant cell elongation.

POS-TUE-177

A NOVEL CIRCADIAN CLOCK REGULATOR IN ARABIDOPSIS

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The circadian clock is an endogenous timing mechanism found in all organisms studied to date regulating many aspects of their behavior, metabolism and physiology. In Arabidopsis, transcriptional networks controlling various biological processes are clock-regulated. Robust rhythms, enhanced fitness and growth vigor are conferred when daily oscillations and clock controlled physiological responses synchronize with environmental conditions. However, the lack of mechanistic knowledge on direct regulatory connections between existing clock components at the transcription level suggests that new clock genes are still missing. To date every clock gene identified in Arabidopsis belongs to a multi-gene family, limiting the use of forward genetic screens due to genetic redundancy. We have taken a reverse genetics approach to identify new clock regulators using a comprehensive library of ~2000 predicted transcription factors (TF) in Arabidopsis to perform a large scale yeast one hybrid screen against the promoter of the core clock gene CCA1. We identified a bHLH transcription factor (bHLH-TF01) that binds to the promoter and negatively regulates the expression of CCA1. Further preliminary data suggest that the effect of bHLH-TF01 on CCA1 is enhanced at high temperature, suggesting a direct mechanistic link to temperature input to the circadian clock. In addition, consistent with the mechanistic model of feedback loops observed in clock gene regulation, we also observed a direct regulation of bHLH-TF01 expression by CCA1, adding a new circuit to the circadian clock network.

POS-WED-178

INFLORESCENCE STEM GRAFTING MADE EASY IN ARABIDOPSIS

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Plant grafting techniques have deepened our understanding of the signals facilitating communication between root and shoot, as well as between shoot and reproductive organs. Transmissible signalling molecules can include hormones, peptides, proteins and metabolites some of which travel long distances to communicate stress, nutrient status, disease and developmental events. While hypocotyl micrografting techniques have been successfully established in Arabidopsis to explore root to shoot communications, inflorescence grafting has not been exploited to the same extent. Two different strategies (horizontal and wedge-style inflorescence grafting) have been developed to explore long distance signalling between the shoot and reproductive organs. In this study a robust wedge-cleft grafting method with success rates greater than 87% has been developed. This method facilitates developing a better tissue contact between the stems from the inflorescence scion and rootstock. The success of grafting was scored using an inflorescence growth assay based upon the growth of primary stem. This method can be successfully used for reproducible translocation experiments to study the physiological, developmental and molecular aspects of long distance signalling. Our simple and reliable method for grafting Arabidopsis inflorescence stem can assist laboratories without grafting experience to explore the molecular and chemical signalling which coordinates communications between the shoot and reproductive tissues (Nisar et al. 2012 Plant Methods 8:50).

POS-TUE-179

THE TETRAPYRROLE MEDIATED PLASTID SIGNAL NEGATIVELY REGULATES *CBF* EXPRESSION UNDER CIRCADIAN CONTROL IN ARABIDOPSIS

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In plant cells, the genetic information is distributed between the nucleus, mitochondria and plastid. Even though the organelles have retained their own genomes, they are dependent on the nucleus to receive the majority of their proteins. In order to coordinate the genome activities, several plastid signals affect the expression of nuclear genes in a process termed retrograde signalling. Stress-induced perturbations of the tetrapyrrole pathway trigger large changes in nuclear gene expression, and the accumulation of Mg-ProtoIX has been shown to be involved in the induction of retrograde signals. These retrograde signals are important during plant stress responses but also facilitate a fine tuning during normal growth conditions. During photoperiodic conditions, cellular fluctuations of Mg-ProtoIX and Mg-ProtoIX-ME correlates with the expression profiles of the nuclear encoded *CBF1-3* genes. The *CBF* genes are up-regulated by cold temperatures to induce a cold response, but under control conditions these genes are regulated by the circadian clock. By using the Mg-ProtoIX and Mg-ProtoIX-ME over-accumulating *crd* mutant, we show that the expression of the CBF transcription factors and their downstream targets *COLD RESPONSIVE 15a* and *47* (*COR15a* and *COR47*) are repressed in response to accumulating tetrapyrrole levels. In addition, the transcription factor ELONGATED HYPOCOTYL 5 mutant (*hy5*) demonstrates an up-regulation of *CBF3* expression specifically and rescues the suppression of *CBF3* expression in the *crdhy5* double mutant. Our results suggest a complex regulation of the *CBF* genes integrating the circadian clock with signals provided by the plastid.

POS-WED-180

FUNCTIONAL ANALYSIS OF GRF9 IN *ARABIDOPSIS THALIANA*

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DNA-binding transcription factors (TFs) are central regulators of essentially all developmental and physiological processes. They bind to *cis*-regulatory elements in promoters of target genes whose expression they regulate, often in concert with other TFs or non-TF regulatory proteins. Our group studies the role of TFs controlling developmental and physiological processes in leaves and unravels the gene regulatory networks controlled by them. The Growth Regulating Factor (GRF) family represents a plant-specific TF family which in *Arabidopsis thaliana* includes nine members. *GRFs* are strongly expressed in actively growing and developing tissues; our work focuses on *GRF9*. To identify its downstream targets we expressed it under the control of an estradiol-inducible promoter in transgenic plants and performed transcriptome analysis, using Affymetrix ATH1 micro-array, 3-6 hours after induction of *GRF9* expression. Candidate target genes that rapidly changed their expression level after *GRF9* induction were analysed further using qRT-PCR. Based on the results obtained, three potential direct *GRF9* targets were selected for further functional and biochemical studies. Using *in vitro* binding site selection, we identified the regulatory *cis*-element to which *GRF9* binds. Currently, upstream regulators controlling the expression of *GRF9* are also unknown. We therefore initiated a deletion study of the *GRF9* promoter aiming at the identification of *cis*-regulatory elements that control *GRF9* expression. Results will be presented.

POS-TUE-181

ARABIDOPSIS SEED GERMINATION REQUIRES GA SIGNALLING IN THE EPIDERMIS THROUGH AN INTERPLAY BETWEEN DELLA AND A *CIS*-ELEMENT TARGETED BY A HOMEODOMAIN TRANSCRIPTION FACTOR

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Appropriate timing for seed germination requires integration of environmental signals into a given genetic background, which modulate hormone levels and signalling. ABA and GAs are two of the most important hormones for germination and have antagonistic roles. GAs stimulates weakening of the embryo surrounding tissues as well as embryo expansion. Molecular mechanisms linking GA perception and signalling in the elongating embryo have not been reported. Moreover, GA responsive elements have been found enriched in gene promoters with increased expression during seed germination only in monocot seeds. We have identified and characterized a 8-bp GA-responsive *cis*-element enriched in Arabidopsis (dicot) gene promoters induced by GA and repressed by DELLAs during seed imbibition. By using an arrayed library containing 1,200 Arabidopsis transcription factors, we identified a homeodomain transcription factor (TF) that specifically binds the *cis*-element and is expressed only in the embryo epidermis during seed imbibition. When GA-signalling is blocked in the epidermis, germination is delayed and expression of epidermis-specific gene promoters containing the *cis*-element is reduced as opposite to what is observed in a *ko-della* mutant. We found that the TF physically interacts with a DELLA protein and the latter is also expressed exclusively in the embryo epidermis during seed imbibition. Results from ongoing experiments suggest that silencing the TF expression recapitulates the molecular and germination phenotypes previously observed by blocking GA-signalling in the epidermis. Our results provide genetic, physiological and molecular evidence that demonstrate that seed germination in Arabidopsis requires GA signalling in the epidermis and suggest that it is controlled by a novel DELLA-TF interaction mediating the induction of the TF target genes.

POS-WED-182

ROLE OF A GATA-TYPE TRANSCRIPTION FACTOR IN REGULATING SEED DORMANCY DOWNSTREAM OF THE DELLA PROTEIN, RGL2

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Seed biology is one of the most widely studied fields in plant physiology because of its immense significance in agriculture. Despite that, most mechanisms regulating seed germination and dormancy are not fully understood. Seed germination is under the tight control of phytohormones, gibberellic acid (GA) and abscisic acid (ABA), the levels of which determine the switch from dormancy to germination under favourable environmental conditions. One of the key transcriptional repressors of seed germination is the DELLA protein, RGA-Like 2 (RGL2). Piskurewicz *et al*., (2008, 2009), provide evidence that there is crosstalk between GA signalling and ABA signalling and that RGL2 appears to serve a key role in this process in *Arabidopsis thaliana*. Based on an earlier microarray data, we identified a GATA-type zinc finger transcription factor (GATAZF) as one of the downstream targets of RGL2 protein. We found that *gatazf* mutant seeds have reduced dormancy than WT while over-expression lines show enhanced dormancy. *GATAZF* transcript levels reduce dramatically with dry after-ripening of seeds and are also negatively regulated by GA. Chromatin immunoprecipitation studies show that RGL2 complex binds to the promoter of *GATAZF* and regulates the gene. Thus, the characterization of this downstream target will help to better understand the mechanism underlying seed germination downstream of GA and RGL2 in seed germination.

POS-TUE-183

THE ROLE OF CALCIUM IN THE NITRATE SIGNALING PATHWAY IN ARABIDOPSIS THALIANA ROOTS

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Nitrate is the main nitrogen source in agriculture soils. Besides its role as a nutrient, nitrate act as a potent signal that control global gene expression. However, the signal transduction pathway involved in the nitrate response still remains elusive. It is known that calcium is an essential second messenger in signal transduction in plants. Nonetheless, its role mediating the response to nitrate has not been addressed. As a first step to determine if calcium is involved in the nitrate response, we tested whether nitrate produced an increased in cytoplasmic calcium concentration. We demonstrated that nitrate treatments produce a transient increase in cytoplasmic calcium. Moreover, a pharmacological inhibitor of phospholipase C (PLC) affected the increase of cytoplasmic calcium. We also evaluated the expression of sentinel nitrate responding genes in Arabidopsis roots in the presence of calcium channel blockers, calcium chelators and a pharmacological inhibitor of PLC. We found that both calcium and PLC are necessary for the expression of such genes in response to nitrate treatments. With this work, we identify a new signaling pathway involving calcium and PLC that modulates changes in gene expression in response to nitrate.
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POS-WED-184

CONTROL OF STARCH ACCUMULATION IN ARABIDOPSIS BY A P-STARVATION INDUCED PEPTIDE-CODING ORPHAN TRANSCRIPT

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A set of ~600 *Arabidopsis* orphan transcripts (*AtoRNAs*), encoding mostly transcripts with limited or without coding capacity, was examined by quantitative real-time PCR for their response to phosphorus (P) limitation. For one of several P-starvation induced *AtoRNAs* that were characterized in more detail, a biological function was established. When over-expressed in P-replete conditions, this *AtoRNA* recapitulated several familiar molecular and physiological P-limitation phenotypes, including e.g. increased anthocyanin content and strong starch accumulation. Overexpression also resulted in high expression of a G6P/Pi translocator gene (*GPT2*), increased levels of major phosphorylated sugars, strongly reduced Krebs cycle intermediates, and increased flux of C^{14}-labelled sugars towards starch. The *AtoRNA* was found to have a P-starvation inducible potential ortholog in *Brassica*. Both transcripts contain predicted small open reading frames encoding highly homologous peptides, which, based on annotation features, could be involved in receptor kinase signaling. The effect on starch accumulation of the disruption of the small open reading frame by an early stop codon and the accumulation of further mutations, that change highly conserved cysteine and serine residues, are being investigated, in order to sustain the idea of the encoded peptide being a biological active molecule. Complementarily, different proteomic approaches are being carried out to identify and characterize the predicted peptide in *Arabidopsis* and *Nicotiana benthamiana*.

POS-TUE-185

CELL TYPE-SPECIFIC AND CONDITION-SENSITIVE ALTERNATIVE SPLICING IN ARABIDOPSIS ROOTS

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Cell type-specific and condition-sensitive pre-mRNA splicing pattern were investigated by mapping the transcriptome of root hairs or nutrient starved whole roots using the newly developed software toolbox RACKJ (http://rackj.sourceforge.net). The percentage of alternatively spliced genes was substantially higher when plants were grown on media deprived of iron or phosphorous. A small, but random overlap of differentially expressed genes and those with growth type-induced changes in pre mRNA splicing indicates that alternative splicing and differential gene expression represent parallel, but potentially interacting regulatory mechanisms. Among the mRNAs with Fe deficiency-induced retained introns, several transcripts with crucial functions in Fe uptake and homeostasis were found, suggesting different regulatory circuits that control protein levels. A comparison with a data set from phosphate-deficient plants revealed that changes in splicing patterns are nutrient-specific. Cell type-specific splicing patterns were investigated by analyzing the transcriptome of Arabidopsis root hair cells, generated by paired-end RNA-seq from protoplasts of plants containing a pEXP7-GFP reporter construct. In root hairs, the population of alternatively spliced transcripts only partly overlapped with those of cells isolated from non-GFP cells (all root cells but root hairs), supporting the assumption that alternative splicing is cell type-specific. Notably, alternative splicing was less complex in root hair cells. In sum, our integrated analysis identified extensive post-transcriptional control, biasing the abundance and activity of proteins in a cell type- and condition-dependent manner. The changes in splicing pattern are stress- and cell type-specific, arguing against the assumption that stochastic fluctuations are the main cause for the generation of alternatively spliced transcripts. The production of a mixture of functional and non-functional transcript may provide a means to fine-tune the abundance of transcripts with critical importance without going to a much slower and, in terms of energy, more costly cycle of protein production and degradation to adjust the level of protein.

POS-WED-186

REGULATION OF THE XERO2 GENE IN *ARABIDOPSIS*

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XERO2 is a plant dehydrin found in the model plant Arabidopsis. The XERO2 dehydrin is strongly expressed in response to cold, dehydration and salt in vascular and reproductive tissues. Overexpression of such dehydrins has been shown to increase cold and drought tolerance. This tolerance may be via their role as membrane stabilizers. Crop losses due to frost damage could be reduced through the increased expression of dehydrin proteins in crop species. Therefore understanding the factors activating *XERO2*, at the genetic level, is of importance for cold-signaling research and improved crop frost tolerance. Microarray analysis is a method commonly used to analyse the genetic changes which occur in response to a stimuli or genetic mutation. The aim of our Microarray experiment was to determine the differences in gene expression occurring in response to cold. We used Arabidopsis samples which were exposed to low temperature and compared these to untreated samples. A large number of genes were found to be up- or down-regulated in the early stages of cold exposure, some encoding transcription factors which potentially bind the MYC sequence present in the *XERO2* promoter. T-DNA mutants of genes identified from the microarray were obtained, and the effect this mutation may have on *XERO2* expression are being examined through the use of promoter:GUS and qRT-PCR analysis. The aim of this work is to identify some of the regulatory proteins (transcription factors) and the pathway(s) activating XERO2.

POS-TUE-187

IDENTIFICATION AND CHARACTERIZATION OF THERMOMEMORY-TRANSCRIPTIONAL REGULATORS

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

POS-WED-188

A NOVEL GLYPHOSATE RESISTANT MUTANT SHEDS LIGHT ON THE REGULATION OF THE SHIKIMATE PATHWAY

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Glyphosate is a broad-spectrum systemic herbicide that competitively inhibits the penultimate enzyme, 5-enolpyruvyl shikimate 3-phosphate synthase (EPSPS), of the shikimate pathway. A glyphosate resistant Arabidopsis mutant (*gre1*) was isolated and genetic analyses indicated a dysfunctional red (R) and far-red (FR) light receptor (phyB) and this is consistent with enhancement of glyphosate sensitivity and glyphosate induced *in planta* shikimate accumulation of the wild type (wt) in FR light. Transcript levels of the shikimate pathway enzymes are up-regulated similarly by R light in both wt and *gre1* but down-regulation by FR light occurs only in the wt. Furthermore, we demonstrate that the first and the forth gene of the shikimate pathway, *DHS1* and *SK1*, contain morning and night responsive circadian clock associated transcription factor-binding sites and that transcript levels show circadian oscillations, suggesting that the shikimate pathway is not only regulated by light quality but also the circadian clock. In addition, the promoters of two genes of the pathway, *DHQS* and *EPSPS1*, harbor phytochrome interaction factor (PIF) binding-sites enabling phyB- dependent repression in the light, resulting in a transcript peak the end of the night in the wt. In contrast, in the mutant the peak phase extends into the dark phase. This shift in the transcriptional program is a consequence of the defect phyB, and that in turn causes a reduction in shikimate accumulation and thereby decreasing the efficiency of glyphosate.

POS-TUE-189

CLV3 PEPTIDE-INDUCED COMPLEX FORMATION BETWEEN THE STEM CELL REGULATORS CLV1 AND CRN

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The establishment and maintenance of the *Arabidopsis thaliana* stem cell niche is controlled through a negative feedback loop, comprising among others, the leucin-rich repeat (LRR) receptor-like kinase CLAVATA1 (CLV1), the LRR receptor-like protein CLAVATA2 (CLV2) and the protein kinase CORYNE (CRN). The signaling peptide CLAVATA3 (CLV3) represents a negative regulator of stem cell fate and was shown to bind to the LRR receptor domain of CLV1, thereby triggering downstream events that lead to a restriction of stem cell fate. While several signaling peptides and receptors that could play a role in this pathway have been identified, little is known about the molecular mechanisms underlying the signal transduction. The techniques offered by modern fluorescence microscopy are powerful tools to gain insight into these mechanisms. Utilizing these tools we were able to show that multimeric receptor complexes are formed as a result of the peptide being bound by its receptor.

POS-WED-190

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF NOVEL TRANSCRIPTION FACTORS WHICH ARE PHOSPHORYLATED IN RESPONSE TO ABSCISIC ACID IN GUARD CELLS

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Stomata open in response to light and close following exposure to abscisic acid (ABA). ABA signaling begins at ABA receptors that induce protein phosphorylation, however, the downstream events in guard cells are still unclear. In this study, we detected *in vivo* substrates of ABA-responsive protein kinases in Arabidopsis guard cell protoplasts by protein-blot analysis with a 14-3-3 protein, and found that 43- and 53-kDa proteins were phosphorylated in response to ABA in a guard cell specific manner. By LC-MS/MS analyses, we demonstrated that they are three novel basic helix-loop-helix (bHLH) transcription factors, and designated them as AKSs (ABA-RESPONSIVE KINASE SUBSTRATES; AKS1, AKS2, AKS3). In *aks1aks2-1* double mutants, stomatal opening by light was slowed down, but ABA-mediated stomatal closure was not affected, suggesting that AKS1 and AKS2 act as enhancers of stomatal opening. To clarify the functional role of AKS proteins, we investigated the activities of the components involved in stomatal opening in the mutant. We found that the K^+ uptake in response to light, the activity of the K^+_{in} channel, and the transcript amount of major K^+_{in} channels were reduced in the mutant guard cells, while the plasma membrane H^+-ATPase activity was unchanged. Chromatin immunoprecipitation assays revealed that AKS1 bound directly to the promoter of *KAT1* and that the binding was released by ABA-induced phosphorylation. Our results demonstrated that AKSs facilitate stomatal opening via transcription and/or regulation of K^+_{in} channels, and that ABA negatively affects K^+_{in} channel activity by phosphorylation of the factors.

POS-TUE-191

ENHANCED RESISTANCE TO DIVERSE PATHOGENS AND PESTS CONFERRED BY MUTATIONS IN GLUTATHIONE S-TRANSFERASE SIGNALLING PATHWAYS

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Glutathione S-transferases (GST) perform key roles in protecting tissues from oxidative damage or toxic products. In plants, transcriptional activation of *GSTs* can be induced by a range of signals including hormones, herbicides and pathogen attack, with *GSTF8* commonly used as a marker gene for early stress and defense responses. Characterising the transcriptional response of *GSTF8*, we identified several novel chemically induced mutants with constitutive *GSTF8* promoter activity and which confer enhanced resistance to diverse pathogens and pests. In particular, one termed *enhanced stress response3 (esr3)* exhibits increased resistance to aphid attack and to specific root or leaf infecting fungal pathogens. These mutants are currently under characterisation through whole transcriptome sequencing (RNAseq), cloning and subsequent functional studies. A previous screen for mutants with a loss of stress inducible *GSTF8* expression identified *disrupted in stress response1 (dsr1)* which exhibits increased susceptibility to specific pathogens and is caused by a mutation within the substrate binding site of the mitochondrial complex II succinate dehyrogenase subunit SDH1-1 (Gleason *et al.* 2011). Thus, proteins involved in regulating *GSTF8* expression play critical roles in plant defense responses. Together, the mutants we are identifying are providing new insight into mechanisms of plant defense and stress responses which can ultimately be manipulated to reduce crop losses caused by biotic and abiotic factors.

POS-WED-192

THE N-END RULE PATHWAY MEDIATES NITRIC OXIDE SENSING

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Nitric Oxide (NO) is a small ubiquitous molecule that functions as an important signalling compound in both prokaryotes and eukaryotes. Although some responses to NO involve S-nitrosylation of regulatory proteins, a general mechanism for NO sensing has not been identified in plants. Here we show that the N-end rule pathway of targeted proteolysis acts as a general sensor of NO in plants through targeted destruction of protein substrates with N-terminal Cysteine (Nt-Cys). Plants lacking N-end rule pathway components accumulate Nt-Cys substrates, which are also stabilised under reduced NO. We show for the first time a key role for NO in the control of plant perception of oxygen, and demonstrate NO-dependent degradation of the Group VII ERF transcription factor HYPOXIA RESONSIVE2 (HRE2). We identify the constitutively expressed Group VII ERFs RAP2.12, RAP2.2 and RAP2.3 as central regulators of seed germination via NO sensing through the N-end rule pathway. We define the molecular mechanism through which they enhance sensitivity to ABA, by activating expression of the *ABA INSENSITIVE5* (*ABI5*) gene, which controls seed ABA responses. Furthermore, we demonstrate that stomatal closure is also controlled through the N-end rule pathway. Our work shows that plants use the N-end rule pathway as a sensor for NO, providing evidence of an evolutionarily ancient origin for this mechanism, and highlight the role of Group VII ERFs as central hubs mediating signalling crosstalk.

POS-TUE-193

INVESTIGATING ROLES FOR AT3G04420 AND AT1G33060, TWO NAC-DOMAIN TRANSCRIPTION FACTORS, AS MASTER REGULATORS OF TRANSFER CELL DEVELOPMENT IN *ARABIDOPSIS THALIANA*

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Transfer cells (TCs) are anatomically specialized with elaborate wall ingrowths which enlarge plasma membrane surface area for increased nutrient transport functions. TCs therefore perform important roles in plant development, but little is known of the genetic pathways which regulate wall ingrowth formation, a unique example of highly localized wall deposition in plants. Members of the NAC-domain family of transcription factors (TFs), such as VND6 and VND7, function as master regulators of secondary wall deposition, a process which in some cell types such as xylem elements involves highly localized wall deposition. We therefore investigated whether NAC-domain TFs function in regulating transcriptional activation required for biosynthesis of wall ingrowths. Bioinformatics identified 20 NAC-domain genes which show expression characteristics consistent with potential roles as regulators of wall ingrowth deposition in phloem parenchyma (PP) TCs in leaf veins of Arabidopsis. Phenotypic analysis of relevant insertional mutants identified two previously uncharacterized NAC-domain genes, At3g04420 and At1g33060, as putative regulators of this process in PP TCs. Phylogenetic analysis shows that both genes cluster in different sub-clades of the NAC-domain gene family in Arabidopsis, and *in silico* expression analysis shows that both are expressed in leaf tissue but at very low levels, an observation confirmed by qPCR. Complementation and over-expression analysis of these genes will be reported, using both constitutive (*CaMV-35S*) and PP-specific (*AtSWEET11*) promoters. Identification of the downstream targets of these NAC-domain TFs is also underway.

POS-WED-194

SYSTEMS APPROACHES MAP REGULATORY NETWORKS DOWNSTREAM OF THE AUXIN RECEPTOR AFB3 IN THE NITRATE RESPONSE OF *ARABIDOPSIS THALIANA* ROOTS

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Auxin is a key phytohormone regulating central processes in plants. Although the mechanism by which auxin triggers changes in gene expression is well understood, little is known about the specific role of the individual members of the TIR1/AFB receptors, the Aux/IAA repressors and the ARF transcription factors and/or the molecular pathways acting downstream leading to plant responses to the environment. We have previously shown that AFB3 has a role in coordinating primary and lateral root growth to nitrate availability. In this work, we used an integrated genomics, bioinformatics and molecular genetics approach to dissect regulatory networks acting downstream of AFB3 that are activated by nitrate in roots. We found that the NAC4 transcription factor is a key regulatory element controlling a nitrate-responsive network. We found that *nac4* mutants have altered lateral root growth but normal primary root growth in response to nitrate. This result suggests AFB3 is able to activate two independent pathways to control root system architecture. Our systems approach unraveled key components of the AFB3 regulatory network leading to changes in lateral root growth in response to nitrate. This work was funded by the International Early Career Scientist program form Howard Hughes Medical Institute (55007421), FONDAP Center for Genome Regulation (1509007), Millennium Nucleus Center for Plant Functional Genomics (P10-062-F), FONDECYT 1100698, ANR-CONICYT program (ANR-07) and CORFO Genome Program (CORFO07Genoma01). E.A.V. is supported by Proyecto de Insercion en la Academia (PSD74).

POS-TUE-195

CIS-CAROTENES: DO THEY HAVE REGULATORY ROLES IN PLANTS?

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Carotenoids are essential in all photosynthetic organisms and promote animal health. *Cis*-carotenes are intermediate metabolites of the carotenoid pathway and their function(s) in plants is yet to be discovered. *Cis*-carotenes are hypothesised to mediate chloroplast to nuclear communication by controlling nuclear gene expression. Interestingly, plant carotenogenesis requires four independent enzymes to desaturate and isomerase phytoene to *all-trans*-lycopene in the upper half of the carotenoid biosynthesis pathway; whilst in bacteria the carotenoid pathway requires only one enzyme, phytoene desaturase (CrtI). The loss-of-function of the *CAROTENOID ISOMERASE* (CRTISO) leads to an over accumulation of *cis*-carotenes in dark grown tissues as well as a range of other interesting phenotypes with respect to chloroplast and plant development. In this study we tested the hypothesis that *cis*-carotene(s) are involved in signalling perturbations in chloroplast development and can influence plant development. Transgenic carotenoid mutants overexpressing a bacterial *CrtI* gene from *Pantoa agglomerans* were generated. A norflurazon-based assay was developed to select for transgenic lines overexpressing CrtI and essentially uncouple *cis*-carotene biosynthesis from the accumulation of carotenoids required for photosynthesis. Results to date have revealed specific *cis*-carotenes that could signal chloroplast to nucleus communication and their function in controlling plant development will be presented.

POS-WED-196

ATIPK2β REGULATES FLOWERING TIME THROUGH THE AUTONOMOUS PATHWAY

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Flowering time in Arabidopsis is regulated by environmentally controlled pathways and internal signals. The four major pathways including photoperiod, gibberellin, vernalization and autonomous pathways are integrated by a set of regulators. The *FLOWERING LOCUS C* (*FLC*) is a key gene of vernalization and autonomous pathways. It encodes a MADS domain-containing transcription inhibitor of floral activator genes, which includes *FLOWERING LOCUS T* (*FT*) and *SUPPRESSION OF OVEREXPRESSION OF CO1* (*SOC1*). The Arabidopsis inositol polyphosphate 6-/3-kinase gene (*AtIpk2β*) encoding a key enzyme in phosphatidylinositol (PI) metabolism, plays a role in plant development and response to external stimuli. However, its molecular mechanism in the regulation of flowering time is unclear. Here we report that *AtIPK2β* modulates flowering through autonomous pathway. The T-DNA insertion knockout mutant, *atipk2β*, flowers earlier than the wide-type (WT) under both long-day and short-day conditions. In contrast, the transgenic plants overexpressing *AtIPK2β* flower later compared to WT. Consistent with these findings, the expression of *FT* and *SOC1* is promoted in *atipk2β*, as the transcription level of *FLC* is down-regulated. Additionally, vernalization treatment had no effect on flowering time of the mutant, which suggests that early-flowering of *atipk2β* is not mediated by this pathway. Further analysis demonstrated that AtIPK2β may bind to the chromatin of *FLC* by interacting with a regulator in autonomous pathway. Moreover, the deposition of the repressive H3K27me3 mark on *FLC* locus is increased in *atipk2β*. Taken together, our work indicates a possible role for AtIPK2β as a repressor of flowering time in autonomous pathway by binding to the chromatin of *FLC* and reducing the accumulation of H3K27me3 mark on this locus.

POS-TUE-197

THE *ATMYB80* TRANSCRIPTION FACTOR, ITS DIRECT TARGET GENES AND THE COTTON HOMOLOG *GHMYB80* IN ARABIDOPSIS MALE FERTILITY

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The *AtMYB80* gene encoding a R2R3 MYB transcription factor is expressed in the tapetum and developing microspores from anther stages 5 to 9. The *atmyb80* mutant shows complete male sterility. Function of the MYB80 domains, including the MYB domain, the variable and the conserved regions between MYB80 homologs, was examined using the truncated *AtMYB80* constructs. Diverse roles were shown for the truncation proteins in the *atmyb80* mutant. Three *AtMYB80* direct target genes, encoding a pectin methylesterase, a MYB transcription factor and a glyoxal oxidase (GLOX2), were identified using the chromatin immunoprecipitation (ChIP)-qPCR technique. Electrophoretic mobility shift assays confirmed that MYB80 binds to the three MYB binding elements in the *GLOX2* promoter. Homologs of *AtMYB80* are highly conserved among crop species, including rice (*OsMYB80*), wheat (*TaMYB80*), canola (*BnMYB80*) and cotton (*GhMYB80*). Two *GhMYB80* ortholog genes in *Gossypium hirsutum* were isolated and cloned. The expression pattern of the *GhMYB80* promoter in Arabidopsis was examined using the *GUS* reporter gene and similar result to that of *AtMYB80* was obtained. The full-length *GhMYB80* gene driven by the *AtMYB80* promoter was able to rescue male fertility in the atmyb80 mutant. The *GhMYB80* gene fused with the *32R* repressive motif inhibited the expression of *AtMYB80* target genes, resulting in partial male sterility in wild-type Arabidopsis. This project studies the roles of *AtMYB80* in regulating genes involved in anther/ pollen development. Knowledge gained from its pathway has been using to develop a reversible male sterility system for hybrid seed production.

POS-WED-198

STABLE INTERNAL REFERENCE GENES FOR NORMALIZATION OF REAL-TIME RT-PCR IN TOBACCO *(NICOTIANA TABACUM)* DURING VIRUS INFECTION

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Real-time RT-PCR is a powerful technique for the measurement of gene expression, but its accuracy depends on the stability of the internal reference gene(s) used for data normalization. Tobacco (*Nicotiana tabacum*) is an important species in studies of plant gene expression, but stable reference genes have not been well-studied in this system. We address this issue by analyzing the expression stability of eight potential tobacco reference genes. Primers targeting each gene (*18S rRNA*, *EF-1α*, *Ntubc2*, *β-tubulin*, *PP2A*, *L25*, *Actin* and *NTCP-23*) were developed and optimized. The expression of each gene then was measured by real-time RT-PCR of tobacco cDNAs derived from RNAs extracted from tobacco plants infected by tobacco mosaic virus (TMV), cucumber mosaic virus, potato virus X and potato virus Y. Overall, *PP2A* and *Ntubc2* demonstrated the highest expression stability, followed by *Actin*. Measurement of *PP2A* and *Ntubc2* was sufficient for accurate normalization in virus-infected tobacco. Although the average reference gene transcript stability examined for four viruses was comparable by both geNorm and NormFinder analyses for the extremes (most stable and least stable), individual viruses showed different orders of reference gene stability. Stimulation of defense genes in *N* gene tobacco after TMV infection was verified using these reference genes, and all techniques were optimized to enable a high-throughput approach. Excluding the two least stable reference genes (*NTCP-23* and *Actin*), the levels of mRNAs encoded by four different defense genes (*ERF5*, *MYB1*, *RDR1* and *IVR*) at 24 hpi by TMV were generally similar for the other reference genes used. These results provide a foundation for the more accurate and widespread use of real-time RT-PCR in tobacco.

POS-TUE-199

CHARACTERISATION OF PUTATIVE PLASMODESMATA PROTEINS OF THE ARABIDOPSIS CALNEXIN FAMILY

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Plasmodesmata are plasma membrane-lined channels spanning the cell wall, connecting the cytoplasm and endoplasmic reticulum (ER) of adjacent cells. Plant survival depends upon the proper function and regulation of plasmodesmata, since developmental signals, nutrients, and even viruses move through these channels. However, only a few protein constituents of plasmodesmata have been discovered. In this study, we extend a comparative proteomics analysis of Chara (Faulkner et al., Proteomics 5: 2866) by identifying and characterising Arabidopsis proteins with sequence similarity to characean peptides isolated from plasmodesmata-rich cell fractions. These proteins were screened for plasmodesmatal localisation in Arabidopsis and Nicotiana benthamiana using green fluorescent protein (GFP) tagging. Calnexin, one of the proteins identified, is a type I membrane protein localised in the ER with the main catalytic domain lying within the ER lumen where it may function as a chaperone. The Arabidopsis calnexin protein family consists of two members, AtCNX1 and AtCNX2, with 83% amino acid identity. Both proteins colocalise with aniline blue-induced fluorescence of callose at plasmodesmata, and colocalisation is reduced upon deletion of the signal peptide. Although no gross morphological differences were observed, cell-to-cell diffusion of GFP as well as deposition of callose at plasmodesmata were affected in knock-out mutants, suggesting some role for calnexin in plasmodesmatal physiology. Quantitative real-time PCR data suggested that in mutants, other chaperones may provide redundancy for CNX, including calreticulin, an ER-lumenal homolog of calnexin. By inference from mutant plasmodesmatal phenotype, CNX1 and CNX2 may play a role in regulating plasmodesmatal aperture by enabling normal callose accumulation in wild-types.

POS-WED-200

PHOSPHOROTHIOATE ANTISENSE OLIGODEOXYNUCLEOTIDES TO TRANSIENTLY SUPPRESS GENE EXPRESSION IN LIVING POLLEN TUBES

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Sexual reproduction is an essential biological event for proliferation of plants. The pollen tube (PT) containing male gametes elongates and penetrates into the pistils for successful fertilization. The molecular mechanisms of a cell-cell communication between PTs and pistil remain largely unknown. Here, we developed a transient gene knockdown by phosphorothioate antisense oligodeoxynucleotide (AS-ODN) without cytofectin, which is a simple and easy tool to transiently suppress the gene expression in *Arabidopsis thaliana* living PTs. During the optimization of the assay, we found that pollen germination and PT growth were dependent on both the agarose and the prehydration of pollen grains. The PTs treated with AS-ODN against both *ANX1* and *ANX2* showed knotted-, blanching and ruptured morphology *in vitro*/semi-*in vitro*, whereas with AS-ODN against *ROP1* and *CalS5* showed waving and short PTs *in vitro*, respectively. These AS-ODNs also affected pollen germination rate and pollen tube length. PT growth was impaired in a dose-dependent manner of AS-ODN, more than 10 μM. The expression level of both *ANX1* and *ANX2* in PTs treated with their AS-ODN were similar to in its sense control, which indicates the inhibition was not directly related to cleavage both *ANX1* and *ANX2* mRNA. This method would enable us to make a novel high-throughput application to identify reproductively important genes in *Arabidopsis*.

POS-TUE-201

SHORT- AND LONG-DISTANCE SIGNAL TRANSMISSION – FOLLOWING TAGGED PROTEINS AND THEIR MRNA ACROSS GRAFT JUNCTIONS IN ARABIDOPSIS

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We investigated the capacity of the phloem in *Arabidopsis thaliana* hypocotyls to carry macromolecules both shootward from roots and rootward from shoots. Rootstocks or scions (shoots) expressing tagged proteins were grafted in the hypocotyl to non-expressing scions or rootstocks, respectively. We traced protein and mRNA from tissues expressing either green fluorescent protein (GFP), glucoronidase (GUS), or larger GFP-tagged proteins moving from transgenic scions to wild-type (WT) rootstocks, or vice versa. Free GFP (27 kDa mwt), expressed either throughout the tissue or only in phloem companion cells, moved rapidly via reconnected phloem from GFP-expressing scions to WT root tips, but moved only slowly from GFP-expressing rootstocks into the phloem of WT scions. Larger proteins such as GFP-sporamin (67 kDa) or GUS (68 kDa) also moved readily in the phloem from scion tissue into WT roots, but in WT scions on transgenic rootstocks, protein was detected in only a few phloem sieve elements close to the graft junction. GFP associated with the endoplasmic reticulum rarely crossed the graft junction in either direction, and was detected within 2-4 cells of the junction. Similarly, transgene mRNA from scions was readily detected in WT rootstocks, but transgene mRNA from rootstocks was only detected in WT scion tissue very close to the graft junction. Clearly, non-native proteins and mRNA can move bidirectionally in phloem, although there is substantially greater movement with mass flow. Tracer proteins were found only in phloem in the destination WT tissue and accumulated in companion cells. We speculate that similar to the introduced tracer proteins, many endogenous macromolecules may be able to move bidirectionally once they enter the phloem. Escape from the phloem may then require specific unloading signals, and the tight control of unloading may be key to regulation of development by abundant phloem-mobile proteins and RNA species.

POS-WED-202

IDENTIFICATION OF A NEW LIGAND FOR THE HAESA-LIKE2 LEUCINE-RICH REPEAT RECEPTOR-LIKE KINASE

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Peptide ligands are essential players in plant growth and developmental processes. *INFLORESCENCE DEFICIENT IN ABSCISSION (IDA)* encodes a secreted peptide required for cell separation during floral organ abscission and lateral root emergence in Arabidopsis (Butenko et al., Plant Cell, 2003; Kumpf et al., PNAS 2013). Genetic evidence suggests IDA to be the ligand of the two leucine-rich repeat receptor-like kinases (LRR-RLK) HAESA (HAE) and HAESA-LIKE 2 (HSL2), as IDA is dependent on these receptors to exert its function (Stenvik et al., Plant Cell, 2008; Cho et al., PNAS, 2008). IDA belongs to a protein family comprising the IDA-LIKE (IDL) proteins, and recent studies suggest that the active peptides are post-translationallly modified. It has been hypothesized that HSL receptors are the native receptors of the IDL peptides, and responsible for mediating signaling resulting in cell separation in different parts of the plant (Butenko et al., Trends Plant Sci, 2009). Interestingly, both the HSL2 receptor and the IDL1 peptide are expressed in the columella cells in the primary root cap. Here we show that the root phenotype of plants overexpressing IDL1 is abolished in *hsl2* mutant background and that post-translationally modified IDL1 peptides can partake in a direct biochemical interaction with the HSL2 receptor. Thus, we have identifed a new IDL-HSL ligand-receptor pair. Based on recent findings, possible mechanisms of signaling and roles played by this module in the root cap will be discussed.

POS-TUE-203

THE POLLEN-EXPRESSED TRANSCRIPTION FACTOR CUPID FAMILY CONTROLS MALE-FEMALE INTERACTION IN ARABIDOPSIS FERTILIZATION

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The *CUPID* (*CPD*) genes were identified by a manual search of the gene expression profile data of Arabidopsis from TAIR databases (http://www.arabidopsis.org). The family has 7 members coded as CPD1-CPD7. Among them, the CPD1, CPD2 and CPD3 share the highest amino acid sequence identity with each other and are expressed mainly in mature pollen grains and pollen tubes. All of their single mutants and double mutants did not exhibit any visible defective phenotype. The *cpd1 cpd2 cpd3* triple mutants also were not defective in pollen development, pollen germination, pollen tube growth and tube guidance. However, the triple mutant pollen tube could not stop growing and failed to discharge the sperms in embryo sac when it entered the embryo sac. The *cpd1 cpd2 cpd3* triple mutations significantly affect the expression of a group of pollen-expressed genes in mature pollen grains and pollen tubes. The results indicate that the CPDs participate in the pollen tube reception, possibly through controlling the expression of the downstream genes, providing a novel evidence for that male pollen tube factors are involved in pollen tube reception.

POS-WED-204

PROMOTER ANALYSIS OF AN *ARABIDOPSIS* GENE FOR 9-CIS-EPOXYCAROTENOID DIOXYGENASE-3 (*ATNCED3*) INVOLVED IN DEHYDRATION-INDUCIBLE TRANSCRIPTION

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Abscisic acid (ABA) plays important roles in not only physiological responses to dehydration including stomata closure but also induction of many dehydration stress genes. ABA is synthesized de novo in response to dehydration. The major ABA biosynthesis pathway is regulated by 9-cis-epoxy carotenoid dioxygenase (NCED). Among five members of Arabidopsis *NCED* genes, the expression of *AtNCED3* gene is highly induced in response to dehydration and function in stress tolerance. For better understanding of regulatory mechanism of the early stages of the dehydration stress response, it is necessary to analyze the transcriptional regulatory system of *AtNCED3*. Using transgenic plants contained deletion series of the 3 kb *AtNCED3* promoter and fused to *GFP* reporter gene, we identified the promoter region in the 200 bp region at -2.33 kb to -2.13 kb upstream from the coding region. We further analyzed the 200 bp region to show that an overlapping G-box recognition sequence (CACGTG) at -2,248 base pairs (bp) from the transcriptional start site of *AtNCED3* is an important *cis*-acting element in the dehydration responsive induction. We discuss the possible transcriptional regulatory system of dehydration-responsive *AtNCED3* expression, and transcriptional regulation of ABA accumulation under water-deficit conditions.

POS-TUE-205

ACCLIMATION RESPONSES OF *ARABIDOPSIS THALIANA* TO SUSTAINED PHOSPHITE TREATMENTS

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Phosphite ($H_2PO_3^-$, Phi) is an analog of phosphate ($H_2PO_4^-$, Pi) which is not metabolized by plants and therefore accumulates in the tissue. Because of their close steric resemblance Phi is able to mimic Pi, thus impeding Pi sensing and signaling mechanisms. In Pi limited plants Phi inhibits phosphate starvation responses, i.e. adaptations aimed at coping with limited Pi supply and increasing Pi uptake capacity. Thus, Phi has constrictive effects on plant growth under low P supply. Phi is also commercially marketed as a biostat against oomycete pathogens (e.g. *Phytophthora* spp.) for agricultural application and even used on an ecosystems scale. Phi directly inhibits pathogen growth through interference with phosphate-dependent metabolism and/or phosphate signaling which is paralleled in plants grown on high phosphite concentrations. In addition, Phi activates plant defense responses, e.g. by SA-dependent induction of defense gene expression or increased callose deposition, by a yet unknown mode of action. We have characterized the impact of prolonged phosphite treatment by analyses of growth responses, root development, gene expression and metabolic adjustments. We show that at low levels Phi has indirect impact on plant growth by inhibiting phosphate uptake whereas at elevated tissue contents Phi directly impairs growth. Phi is not able to suppress the induction of lateral roots under P limiting conditions and the up-regulation of a set of phosphate starvation induced genes. Furthermore, phosphite induces specific changes in the abundance of several metabolites, especially amino acids, which may have relevance for the understanding of phosphite induced resistance.

POS-WED-206

TRANSCRIPTIONAL REGULATORY CASCADE IN HEAT STRESS RESPONSE OF *ARABIDOPSIS THALIANA*

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Plants as sessile organisms have to dynamically adjust to the changing environment and cope with serious biotic and abiotic stresses. One of such stresses is extensive heat, threatening crops worldwide and usually connected with seasonal drought. In order to survive heat plants have developed various molecular mechanisms allowing perception, direct response and adaptation maximizing survival, growth and reproduction in elevated temperatures. Whereas most of the core heat stress-induced genes have been identified; many genes exhibit conditional heat-stress response, depending on e.g. light conditions, or diurnal and circadian cycle, and the exact regulatory interactions leading to activation of a certain subset of genes in given conditions remain largely unknown. My research is based on results of recent study conducted in our group concerning gene expression time-series data collected during early response of Arabidopsis plants to different temperature and light intensity regimes. Using a bioinformatic approach we have generated a network of putative regulatory interactions during heat stress which include transcription factors and their known and potential gene-targets. The goal of my project is to validate putative targets of selected TFs (HsfA2, DREB2a, HsfA7a, ANAC078, RHL41) and to characterize gene regulatory logic governing gene expression under heat stress. Predicted interactions are validated by qRT-PCR-based analysis of transcription factor knock-out lines exposed to heat stress and by trans-activation assays. Furthermore I focused on 3 major regulatory logic gates: single input gate (SI), "AND" and "OR", and designed an experimental approach allowing to classify observed regulatory interactions as one of these. My results confirm a range of known TF – target interactions and indicate presence of new ones, including a negative feedback loop between HsfA2 and ANAC078.

POS-TUE-207

GENERAL AND DIFFERENTIAL CHANGES IN THE TRANSLATOME PARTICIPATE IN THE ESTABLISHMENT OF THE HEAT STRESS RESPONSE IN *ARABIDOPSIS* SEEDLINGS

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Heat stress is one of the most prominent and deleterious environmental threads affecting plant growth and development. Upon high temperatures, plants launch specialized gene expression programs that promote stress protection and survival. These programs involve global and specific changes at the transcriptional and translational levels; however the coordination of these processes and their specific role in the establishment of the heat stress response is not fully elucidated. We have carried out a genome wide analysis to monitor simultaneously the individual changes in the transcriptional and translational mRNA levels of *Arabidopsis thaliana* seedlings after the exposure to a heat shock stress. Our results demonstrated that, superimposed to transcription, translation exerts a wide but dual regulation of gene expression. For the majority of the mRNAs, translation is severely repressed causing a deep decrease in the association of the bulk of mRNAs to polysomes. However, some relevant mRNAs involved in different aspects of homeostasis maintenance follow a differential pattern of translation. Analysis of the sequence of the differentially translated mRNAs unraveled some special features that take part in the discrimination mechanisms for mRNA polysome loading. Among the identified differential translated genes stand out key regulators of the stress response highlighting the main role of translation in the early establishment of physiological response of plants to elevated temperatures.

POS-WED-208

FOUR TRANSCRIPTION FACTORS ARE RELATED TO THE MULTIPLE RESPONSE OF ARABIDOPSIS ATGST11 GENE UNDER ABIOTIC STRESSES

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Aluminum (Al)-induced Arabidopsis glutathione S-transferase gene (AtGST11) was analyzed to investigate the gene-response mechanism for various abiotic stresses. A GUS fusion gene including 1-kb of the 5'-upstream region (pAtGST11::GUS) was introduced into Ler-0. The fusion gene was actually induced by Al treatment (approximately 8 h for maximum expression). Transgenic lines also showed an increase of GUS activity by cold, heat, metal toxicity, and oxidative damages, suggesting a multiple induction to the stressors tested. And it was suggested that stress dependent transcription factors (TFs) are probably related to the expression. To clarify the response mechanisms, a new approach for the screening of DNA binding proteins, based on a phage display technique (Bio-panning) was introduced. Two candidates were obtained and they were confirmed their binds to the promoter region by electrophoresis mobility shift assay (EMSA). These two were C3HC4type RING finger (DAL1) and Homeobox protein 6 (AtHB6). Recently, another two candidates were isolated, using yeast one hybrid analysis. EMSA showed that both proteins also bound to the 5'-upstream region of AtGST11 gene. They encoded a putative bZIP TF (AtbZIP30) and ethylene response element binding factor 2 (AtERF2). Promoter activity assays indicated that AtbZIP30 and AtERF2 up-regulate AtGST11and DAL1 and AtHB6 down-regulate. Expression of the AtGST11 in Col-0 line was increased by Cd, Cu and Al, but decreased by cold, heat and diamide. To investigate how each TF relates to the gene-regulation of the AtGST11 under these stresses, quantitative real-time PCR was performed. The results indicated that DAL1 and AtHB6 down-regulate the expression of AtGST11 under most of the stresses tested as repressors, and AtERF2 up-regulates under heat stress as a inducer.

POS-TUE-209

ABSCISIC ACID AND AUXIN ANTAGONISTICALLY REGULATE ROOT MERISTEM ACTIVITY THROUGH A NODULIN HOMEOBOX PROTEIN IN ARABIDOPSIS

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Root meristem activity is regulated by different phytohormones mainly as a consequence of their effects on a key auxin repressor, SHORT HYPOCOTYL2 (SHY2/IAA3). However, the molecular mechanism underlying how a stress hormone, abscisic acid (ABA), regulates root growth is largely unknown. Here, we show that mutations in an Arabidopsis nodulin homeobox (NDX) protein lead to increased sensitivity to ABA in inhibiting root growth. The expression of NDX is strongly induced by auxin and suppressed by ABA. NDX regulates the expression of PIN-FORMED1. Under ABA treatment, the ndx mutation reduces auxin transport from the shoot to the root meristem, alleviates the auxin signaling, and reduces the root meristem's size by inhibiting cell division and promoting cell differentiation. Thus, NDX is a novel positive regulator of root meristem activity in Arabidopsis, and its effect is controlled by the interaction between ABA and auxin.

POS-WED-210

FUNCTIONAL AND TRANSCRIPTOME ANALYSIS REVEALS AN ADAPTIVE STRATEGY FOR ABIOTIC STRESS TOLERANCE DEPENDANT ON THE BIFUNCTIONAL ACTIVITY OF MEMBERS OF THE ARABIDOPSIS NF-YA TRANSCRIPTION FACTOR FAMILY

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Nuclear Factor Y (NF-Y) is a heterotrimeric complex formed by NF-YA/NF-YB/NFYC subunits that binds to the CCAAT-box in eukaryotic promoters. In contrast to other organisms in which a single gene encodes each subunit, in plants gene families of over 10 members encode each subunit. We found five members of the Arabidopsis thaliana NF-YA family strongly induced by several stress conditions via transcriptional and miR169-related posttranscriptional mechanisms. Dominant repressor versions of NF-YA2, 3, 7 and 10 allowed the identification of genes directly regulated by the NF-Y complex and revealed that NF-YAs participate in gene regulation via two mechanisms, one depending on binding to the CCAAT-box in the promoter of regulated genes and the another, independent of the CCAAT-box, in which NF-YA prevents the interaction of the NF-YB/YC heterodimer with transcription factors. We also found that overexpression of NF-YA2, 7 and 10 results in dwarf plants with enhanced tolerance to several types of abiotic stresses. These phenotypes are related to alterations in the sucrose/starch balance, cell elongation and ABA responses. These observations suggest that increased NF-YA transcript levels in response to abiotic stress is part of an adaptive response to adverse environmental conditions in which plant growth rate reduction plays a key role.

POS-TUE-211

CHARACTERIZATION OF AN IRON OVER-ACCUMULATING MUTANT OF *ARABIDOPSIS THALIANA*

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Iron is necessary for the maintenance of both plant growth and human health. Plants are the major source of human dietary iron yet they are often limited in iron content. Thus, increasing the ability of plants to acquire iron could have significant effects on plant and human nutrition. After screening over 10,000 mutant *Arabidopsis thaliana* lines, we have identified the first mutant to significantly over-accumulate iron compared to wild type. This EMS mutant, which we call 93699, will potentially provide critical information for understanding how plants store iron. ICP-MS data shows that 93699 accumulates significantly more iron in the seed and shoots than wild type and is very sensitive to exogenous iron supply. Synchrotron X-ray fluorescence spectroscopy also shows increased iron in seeds, roots, and shoots, particularly associated with the vasculature. Despite its increased iron storage, 93699 does not accumulate greater levels of the major iron storage protein ferritin than wild type. 93699 is more tolerant to iron-deficient conditions than wild type, which is demonstrated by its longer roots when plants are grown on iron-deficient medium and better growth on alkaline soil. These phenotypes are supported by a constitutive expression of a characteristic iron-deficiency response including ferric chelate reductase activity and IRT1 expression. Microarray analysis comparing 93699 to wild type demonstrates that 93699 has an altered iron transcriptome. In addition to its altered iron response, 93699 is more tolerant to high levels of Ni, Co, and Cd than wild type. We have mapped the mutation and have shown via complementation that a missense mutation is responsible for the observed phenotypes.

POS-WED-212

THIOREDOXIN H-TYPE HAS DUAL FUNCTIONS THAT MOLECULAR CHAPERONE AND DISULFIDE REDUCTASE IN *ARABIDOPSIS*

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Various thioredoxins (Trxs), redox proteins, have been identified from many species. However, many of the physiological functions played by these proteins remain to be explained. We isolated a high M_r (HMW) form of h-type Trx from the heat-treated cytosolic extracts of Arabidopsis (*Arabidopsis thaliana*) suspension cells and designated it as AtTrx-h3. Using recombinant AtTrx-h3, we found that it forms various protein structures ranging from low and oligomeric protein species to HMW complexes. These are closely associated that AtTrx-h3 has dual functions, which as a disulfide reductase and a molecular chaperones through its molecular structures. The disulfide reductase function is observed predominantly in the low M_r forms, whereas the chaperone function predominates in the HMW complexes. The multimeric structures of AtTrx-h3 are regulated redox status as well as heat shock. Two active cysteine residues in AtTrx-h3 are required for disulfide reductase activity, but not for chaperone function. AtTrx-h3 confers enhanced heat-shock tolerance in *Arabidopsis*, primarily through its chaperone function.

POS-TUE-213

EMERGING ROLES OF RNA CHAPERONES IN STRESS RESPONSE AND DEVELOPMENT OF PLANTS

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RNA chaperones are nonspecific RNA-binding proteins (RBPs) that aid in RNA folding process by preventing RNA misfolding or by resolving misfolded RNA species. In recent years, RNA chaperones are recognized as key regulatory factors in diverse cellular processes, including the growth, development, and stress response of plants. During the last years, we have extensively investigated the biological functions and RNA chaperone activity of many cytoplasmic, nuclear, and chloroplast-localized RBPs during development and stress response of Arabidopsis thaliana. Several family members of glycine-rich RNA binding proteins (GRPs), zinc finger-containing GRPs, cold shock domain proteins, spliceosomal proteins, and RNA helicase have been determined to exhibit RNA chaperone function during the stress response and development of plants. In this presentation, discussed will be the recent progress and novel information on the functional roles of RNA chaperones in the growth, development, and stress responses as well as the importance of posttranscriptional regulation of RNA metabolism in plants. [Supported by grants from NRF and Next-Generation BioGreen21].

POS-WED-214

GROWTH PLATFORM-DEPENDENT AND INDEPENDENT PHENOTYPIC AND METABOLIC RESPONSES OF *ARABIDOPSIS THALIANA* AND ITS HALOPHYTIC RELATIVE, *THELLUNGIELLA SALSUGINEA*, UNDER SALT STRESS

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To investigate the natural salt tolerance of the *Arabidopsis* halophytic relative, *Thellungiella salsuginea*, we performed a phenomics study of leaf growth and development under control and salt stress conditions in an *in vitro* plate system and a pot-based soil system. Growth responses of *Arabidopsis* and *Thellungiella* differed depending upon growth platform. Leaf emergence was affected in a similar way in both species grown *in vitro* but the same effects observed in *Arabidopsis* occurred at higher salt concentrations in *Thellungiella*. Leaf emergence of both species was unaffected on soil at all stress levels. We also unmasked a previously unobserved leaf area reduction of *Thellungiella* even under mild stress on both platforms. Metabolic profiling revealed both growth platform-independent and dependent metabolic responses. For instance, *Thellungiella* exhibited higher citrate and malate levels but constitutively low fumarate, galactinol and raffinose content regardless of growth platform. Such metabolic signatures could reflect core *Thellungiella* stress tolerance mechanisms. Growth platform-specific metabolic differences manifested as repression of accumulation of a number of metabolites in *Thellungiella* in the in vitro system compared to the soil system. These included metabolites known to be involved in stress responses. We surmise that perturbation of C:N ratio by inclusion of sucrose in the in vitro system could be responsible for this metabolic repression. Furthermore, the fact that despite large metabolic changes, *Thellungiella* maintained a salt tolerant phenotype compared to *Arabidopsis* on both growth platforms suggests an inherent adaptive plasticity required for an extremophile lifestyle.

POS-TUE-215

DETERMINING THE EFFECT OF PHOSPHATE SUPPLY ON THE PROTEOME OF *ARABIDOPSIS THALIANA* SHOOTS

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Phosphorus (P) is an essential macronutrient for plant growth and development. Although P is relatively abundant in soils, phosphate (P_i) - the fully oxidized form of P that is utilized by plants - is one of the least available plant nutrients. To overcome the problem of P_i deficiency in agricultural systems, concentrated P_i fertilisers are used extensively. However, overuse of such fertilisers has led to alarming estimates that rock P_i reserves could be depleted in as little as 60-80 years. Therefore there is an urgent need to develop crop plants that can acquire and utilize P_i more efficiently. To do this, a thorough understanding is needed of the signaling pathways that regulate P_i acquisition and use when P_i availability is limited. However, as it is thought that plants are typically P_i deprived, it is of particular importance that we understand how the signals involved in P_i deprivation are down regulated upon P_i re-supply. Proteomic changes between P_i deprived and re-supplied *Arabidopsis* shoots were identified using 2D-PAGE. Assessing total protein, 20 proteins increased significantly in abundance with decreasing P_i supply, while 61 proteins decreased in abundance. Additionally, phosphoprotein staining revealed 19 protein spots whose staining intensity increased significantly with decreased P_i supply and just 2 spots whose staining intensity decreased. Significantly changing protein spots will be identified using ESI-TOF mass spectrometry. These results are expected to provide vital insights into the workings of the P_i signaling pathway and provide protein candidates for further analysis.

POS-WED-216

GENES ENCODING PLANT-SPECIFIC CLASS III PEROXIDASES ARE RESPONSIBLE FOR INCREASED COLD TOLERANCE OF THE BRASSINOSTEROID-INSENSITIVE 1 MUTANT

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We previously reported that one of the brassinosteroidinsensitive mutants, *bri1-9*, showed increased cold tolerance compared with both wild type and BRI1-overexpressing transgenic plants, despite its severe growth retardation. This increased tolerance in *bri1-9* resulted from the constitutively high expression of stress-inducible genes under normal conditions. In this report, we focused on the genes encoding class III plant peroxidases (AtPrxs) because we found that, compared with wild type, *bri1-9* plants contain higher levels of reactive oxygen species (ROS) that are not involved with the activation of NADPH oxidase and show an increased level of expression of a subset of genes encoding class III plant peroxidases. Treatment with a peroxidase inhibitor, salicylhydroxamic acid (SHAM), led to the reduction of cold resistance in *bri1-9*. Among 73 genes that encode *AtPrxs* in Arabidopsis, we selected four (*AtPrx1, AtPrx22, AtPrx39, and AtPrx69*) for further functional analyses in response to cold temperatures. T-DNA insertional knockout mutants showed increased sensitivity to cold stress as measured by leaf damage and ion leakage. In contrast, the overexpression of *AtPrx22, AtPrx39, and AtPrx69* increased cold tolerance in the *BRI1-GFP* plants. Taken together, these results indicate that the appropriate expression of a particular subset of *AtPrx* genes and the resulting higher levels of ROS production are required for the cold tolerance.

POS-TUE-217

ACETIC ACID IS ESSENTIAL FOR DROUGHT TOLERANCE IN PLANTS

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Discovery of a novel plant strategy is essential to solve increasing global environmental issues. To that end, chromatin-based understanding is imperative as chromatin modification is widely connected to gene regulation in eukaryotes. However, only a few direct evidences have been found so far for chromatin regulation in plant stress responses. Here we show that Arabidopsis histone deacetylase HDA6, an epigenetic factor having wide range of functions, also regulates drought stress tolerance by acetate fermentation pathway, a quite novel one of drought response. HDA6 directly binds to and represses acetate fermentation genes under normal condition. In response to drought stress, HDA6 binding gradually detached and histone H4 acetylation levels and gene expressions are upregulated. In hda6 mutant, expression levels of acetate fermentation genes and endogenous acetic acid levels are higher compared with wild type. Furthermore, external application of acetic acid successfully enhances drought tolerance of plants, showing that acetic acid, a simple natural compound, functions as a useful resource for improving drought stress tolerance. Our findings propose the novel plant strategy to survive under environmental stress by epigenetically regulating specific metabolic pathway, and opens up the potential of acetic acid towards agricultural application.

POS-WED-218

THE R2R3 MYB GENE *ATMYB73* NEGATIVELY REGULATES THE EXPRESSION OF *SOS1* AND *SOS3* IN *ARABIDOPSIS* ONLY IN RESPONSE TO HIGH SALINITY

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Environmental insults including high salt, drought and low or high temperatures are often associated with a significant loss of agricultural productivity. Plants have evolved a diverse array of signaling pathways to modulate their development in response to various environmental challenges. Here, we report the characterization of a member of the R2R3-MYB transcription factor family, AtMyb73. The expression of *AtMyb73* was up-regulated by salt stress but not by other stresses. The maximum level of *AtMyb73* expression occurred at 6 hr of 300 mM NaCl treatment. Under salt stress *atmyb73 ko* mutant plants exhibited higher survival rate than Col-0 in salt stress condition. Using qRT-PCR, we determined that the accumulation of *SOS1* and *SOS3* transcripts was higher in *atmyb73 ko* and *atmyb73 eko* plants than in Col-0 plants in response to 300 mM NaCl treatment. These results indicate that *AtMyb73* is a negative regulator of SOS induction only in response to salt stress.

POS-TUE-219

ATDABB1 IS A PATHOGEN-RESPONSIVE PROTEIN WITH ANTIFUNGAL ACTIVITY IN *ARABIDOPSIS THALIANA*

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To purify plant antifungal protein from *Arabidopsis thaliana* leaves, we used anion exchange chromatography and high-performance liquid chromatography. Using MALDI-TOF/MS analysis, we determined the amino acid sequence of purified protein, and found that the sequence matched that of a hypothetical *Arabidopsis* protein in GenBank (accession number NP_175547). We designated the protein as AtDabb1. We cloned the full-length cDNA encoding the hypothetical protein from an *Arabidopsis* leaf cDNA library, the recombinant protein was expressed in *Escherichia coli* and found to significantly inhibit cell growth of various pathogenic fungal strains. The AtDabb1 transcript level was induced by pathogen-related signaling molecules including salicylic acid(SA) and jasmonic acid(JA). We suggest that AtDabb1 may play an important role in an induced defense mechanism against various stress and pathogenic damage.

POS-WED-220

FUNCTIONAL ANALYSIS OF NOVEL CHLOROPLAST MEMBRANE PROTEINS, COR413-IM1 AND COR413-IM2 REGULATED BY AN *ARABIDOPSIS* TRANSCRIPTION FACTOR *DREB1A*

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Plants, being sessile, have the ability to dramatically alter their gene expression patterns in response to environmental changes, such as temperature and water availability. DREB1A/CBF3 strongly up-regulates expression of many cold-inducible genes, indicating that the DREB1A and its downstream genes have important roles in modulating the plant responses to cold stresses. In this study, we identified two cold-regulated (*COR*) genes of unknown function, designated as the COR413 chloroplast inner envelope membrane group (COR413IM1 and COR413IM2), as downstream targets of DREB1A. We analyzed gene expression patterns of *COR413-IM1* and *COR413-IM2* and found these genes were clearly induced after accumulation of *DREB1A* transcripts under cold stress. Evaluation of the expression of a *GUS* reporter gene driven by the *COR413-IM1* and *COR413-IM2* promoters in transgenic plants revealed that induction of these promoters was initiated in aerial plant parts in response to cold-stress treatments. Subcellular localization of COR413-IM1 and COR413-IM2 proteins fused to GFP was observed in chloroplast membranes in plants under cold stress. Bimolecular fluorescence complementation (BiFC) assays suggested that these proteins interact to form homo- or hetero-dimers. We analyzed phenotypes of T-DNA insertion mutants of these genes and found that *cor413im1* and *cor413im2* mutants accumulated more anthocyanin as compared to the wild-type plants under cold-stress conditions. The *im1 im2* double mutants generated by RNAi-mediated gene silencing showed a more drastic phenotype than the single mutants. These results suggested a possible and partial functional redundancy between COR413-IM1 and COR413-IM2 proteins in chloroplasts under cold stress.

POS-TUE-221

HEAT-INDUCED CHAPERONE ACTIVITY OF SERINE / THREONINE PROTEIN PHOSPHATASE 5, PP5, IMPROVES THERMOTOLERANCE IN *ARABIDOPSIS*

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This study reports that *Arabidopsis thaliana* serine / threonine phosphatase 5 (AtPP5) plays a pivotal role in heat stress resistance. A high-molecularweight (HMW) form of AtPP5 was identified from heat-treated *A. thaliana* suspension cells. AtPP5 performs diverse functions, as a protein phosphatase, foldase chaperone, and holdase chaperone. The enzymatic activities of this versatile protein are closely associated with its oligomeric status, ranging from low oligomeric protein species to HMW complexes. The phosphatase and foldase chaperone functions of AtPP5 are associated primarily with the low-molecular-weight (LMW) form, whereas the HMW form shows holdase chaperone activity. AtPP5 over-expressing transgenic conferred enhanced heat shock resistance to wild-type *A. thaliana* and a T-DNA insertion knock-out mutant was sensitive to acquired thermotolerance. A recombinant phosphatase mutant (H290N) showed markedly increased holdase chaperone activity. In addition, increased thermotolerance was observed in H290N overexpressing transgenic plants, which indicates that the holdase chaperone activity of AtPP5 is primarily responsible for AtPP5-mediated thermotolerance. As a result, this study provides the first evidence that AtPP5 performs multiple enzymatic activities that are mediated by conformational changes induced by heat-shock stress.

POS-WED-222

THE INFLUENCE OF TWO MAJOR ANTIOXIDANTS ON STRESS RESPONSES IN PLANTS

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Stress exposure leads to the production of reactive oxygen species (ROS) and plants respond by synthesizing a number of antioxidant compounds. The two major antioxidants, ascorbate (Asc or vitamin C) and the tripeptide glutathione (GSH), are maintained in a generally reduced state and both contribute to responses to ROS. They are both essential multifunctional metabolites important in development, redox homeostasis and signalling during stress responses in plants. Asc and GSH have independent roles in aspects of stress tolerance and there is some evidence that each is able to compensate for the other to some degree. Mutants partially deficient in Asc are more sensitive to salt stress, high light, and ozone while mutants partially deficient in GSH are more sensitive to other stresses such as heavy metals, pathogens and oxidative stress. While it is clear that both Asc and GSH play pivotal roles in plant stress responses and development the extent to which the functions and physiological status of these two antioxidant pools are interdependent remains unclear. The aim of this study is to create mutant combinations partially deficient in both Asc and GSH and observe their effect on tolerance to different environmental stresses. While the partially GSH-deficient mutant is sensitive to heavy metals and oxidative stress the doubly Asc- GSH-deficient mutant has intermediate resistance compared to wildtype and the corresponding GSH-deficient parent plant. Anthocyanin accumulation is influenced by the level of Asc. More interestingly, while the partially Asc-deficient mutant accumulates less anthocyanin during high light acclimation compared to wildtype the doubly Asc- GSH-deficient mutant appears to restore wildtype level of anthocyanin. This study provides further evidence that both Asc and GSH have interdependent roles during stress responses and may provides new insights into the redox control of both antioxidant pools towards stress defences.

POS-TUE-223

THE EFFECTS OF ELEVATED CARBON DIOXIDE AND TEMPERATURE ON MICRORNA EXPRESSION IN ARABIDOPSIS DEVELOPMENT

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Elevated CO_2 concentration ($[CO_2]$) has been shown to increase plant biomass and crop production and tolerance to drought condition, while unusual high seasonal temperatures have significantly lowered biomass- and grain-production in crops and other plants, suggesting a serious problem in global warming, as by 2050, food production must at least double to feed the world's increasing human population (IPCC report, 2007) and to meet the rising demands for biofuels. We are using Arabidopsis thaliana as a model system to investigate the impact of these two conditions on small RNA expressions and the correlated gene expressions and plant phenotypes. Small RNAs function to silence gene expression and regulate genomic DNA methylation, and environmental stresses can alter their expressions to modulate plant phenotypes. Using the small RNA-sequencing method, we identified known and predicted microRNAs (miRNAs) that were changed significantly in expression by either doubling the atmospheric $[CO_2]$ or by increasing temperature 3-6°C. Notably, nearly all CO_2-influenced miRNAs were also affected by elevated temperature. Using the RNA-sequencing method, we determined strong expression correlations between flowering time regulatory miRNAs and their target transcription factors by elevated $[CO_2]$, suggesting a mechanism for a CO_2-induced early flowering phenotype. Similar correlations were also revealed for miRNAs acting in auxin-signaling, stress responses, and potential cell wall carbohydrate synthesis. In addition, we have also identified significant difference in genomic DNA methylation induced by elevated $[CO_2]$, and have identified differentially methylated cytosine positions and regions and their nearby genes. Our results demonstrate that elevated $[CO_2]$ and elevated temperature can signal miRNA expressions to affect Arabidopsis growth and development, and miRNA regulation of flowering time may underlie the onset of flowering affected by increasing CO_2.

POS-WED-224

DUAL FUNCTIONS OF ARABIDOPSIS SULFIREDOXIN: ACTING AS A REDOX-DEPENDENT SULFINIC ACID REDUCTASE AND AS A REDOX-INDEPENDENT NUCLEASE ENZYME

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Among many proteins with cysteine sulfinic acid (cys-SO2H) residues, the sulfinic forms of certain peroxiredoxins (prxs) are selectively reduced by sulfiredoxin (Srx) in the presence of ATP, Mg2+ and a thiol-reductant. All Srx enzymes contain conserved cysteine residues. Here, we have found that the cys72 in AtSrx was essential to reduce the sulfinic acid form of Arabidopsis 2-Cys Prx whereas the Cys mutant form of AtSrx-C72S, in which the active Cys residue (positioned at 72) was replaced by Ser failed to reduce the sulfinic acid form. Also, Srx showed high amino high sequence homology with that of ParB protein having a nuclease activity. We examined the nucleic acid binding and hydrolyzing activity of the recombinant Srx in Arabidopsis (AtSrx) by using different kinds of the DNA substrates. We found that AtSRx interacts with the DNA substrates without showing substrate specificity. Also, we found that all the DNAs were gradually cleaved by the addition of AtSrx showing the DNA hydrolyzing activity of AtSrx and the nuclease activity was enhanced by divalent cations. Furthermore, the Cys mutant form shows no activity difference on the DNA binding and hydrolyzing activities which suggests that the active site Cys residue of AtSrx (Cys72) that is critically required for a sulfinate reductase function does not involve in its nuclease function. Altogether, we clearly demonstrate the dual functions of AtSrx, acting not only as a redox-dependent sulfinic acid reductase but also as a redox-independent nuclease function.

POS-TUE-225

INTEGRATED ANALYSIS (PHYTOHORMONES AND TRANSCRIPTS) OF THE EFFECTS OF HEAT SHOCK STRESS IN ARABIDOPSIS, RICE AND SOYBEAN

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High temperature is an important stress factor that negatively affects plant growth and crop productivity. Many genes are induced under heat shock (HS) stress and work together to increase thermotolerance. The accumulation of HS proteins (HSPs) is assumed to play a central role in the HS response. Integrated analysis has been reported to elucidate phytohormone-to-gene correlations in both model and crop plants, although the effects of HS stress on these correlations in Arabidopsis, rice and soybean plants remain to be clarified. In this study, we performed integrated analysis using HS stress-exposed Arabidopsis, rice and soybean plants and compared characteristics of identified HS-responsive phytohormones and genes. We used LC-MS to measure levels of phytohormones in plants subjected to HS stress treatments and identified 14 phytohormones. CKs and ABA were identified as representative phytohormones in three kinds of plants. Both phytohormones were down-regulated under HS stress conditions. We also performed transcriptome analysis using microarray. HSPs were identified as representative HS-inducible genes in three kinds of plants. In addition, comprehensive promoter sequence analyses demonstrated general characteristics of promoter sequences in HS-inducible promoters. The highly conserved sequences in HS-inducible promoters of Arabidopsis are similar to those of rice and soybean. HS-responsive element is the most highly conserved sequences.

POS-WED-226

EFFECTS OF PROGRESSIVE DROUGHT IN ARABIDOPSIS PLANTS OVEREXPRESSING OR SILENCED FOR THE PATATIN-LIKE GENE *PPLAIIα*

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Lipolytic enzymes play pivotal roles in membrane remodeling and signaling during development and in response to stress. There is considerable information on enzymes acting on the polar head of glycerolipids, namely phospholipases C and D, but much less is known about the enzymes that release fatty acids, designated phospholipases A (PLA) or lipid acyl hydrolases (LAH). Patatin is a vacuolar storage protein present in potato tubers which displays LAH activity towards phospho- and galactolipids. Patatin-like genes from other plants are responsive to a variety of biotic and abiotic stresses. In Arabidopsis there are 10 patatin-like genes, being *pPLAIIα* the major drought-induced gene in leaves. Previous analyses have shown that drought-stressed Arabidopsis plants have a decreased content of leaf lipids, namely galactolipids and preferentially accumulate free polyunsaturated fatty acids. Here we used transformed plants overexpressing (OE) or silenced (AS) for *pPLAIIα* to address the role of this protein in membrane lipid catabolism under drought. Results from leaf and soil water contents, show that they decrease faster in AS plants during the treatment. Accordingly, drought-stressed OE plants show a better photosynthetic performance. Free fatty acids analysis does not show major differences between the lines, under control or stress conditions. The fact that ROS accumulation seems to be higher in stressed AS lines is in agreement with a protective role of pPLAIIα, possibly by removing damaged fatty acids from membrane lipids. Further analyses are required to understand the role of pPLAIIα in vivo.

POS-TUE-227

HEAT- SHOCK DEPENDENT OLIGOMERIC STATUS ALTERS THE FUNCTION OF A PLANT- SPECIFIC THIOREDOXIN- LIKE PROTEIN, ATTDX

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A heat stable and plant thioredoxin (Trx)- like protein, exhibits multiple functions, acting as a disulfide reductase, foldase chaperone, and holdase chaperone the *Arabidopsis* AtTDX. AtTDX activity have 3 tetratricopeptide repeat (TPR) domains and a Trx motif that depends on its oligomeric status. The disulfide reductase and foldase chaperon functions predominates when AtTDX occurs in the low molecular weight (LMW) form, while the holdase chaperon function in high molecular weight(HMW) complexes. Due to the deletion of the TPR domains results in a significant enhancement of AtTDX disulfide reductase activity and a complete loss of the holdase chaperone function, our data commend that the TPR the TPR domains of AtTDX block the chaperone function. The oligomerization status of AtTDX is reversibly regulated by heat shock which causes a change from LMW to HMW complexes with functional switching from a disulfide reductase and foldase chaperone to a holdase chaperone.We showed that the overexpression of AtTDX in *Arabidopsis* significantly enhanced heat shock tolerance of plants generally through its holdase chaperone activity.

POS-WED-228

IDENTIFICATION OF NOVEL SUBCLASS III SNRK2-INTERACTING PROTEINS IN ARABIDOPSIS

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Subclass III SnRK2s (SNF1-related protein kinase 2) play a pivotal role in coordinating cellular responses to water-deficit stresses in plants. In response to water-deficit stresses, subclass III SnRK2s are activated and then modulate activities of downstream targets including channels (e.g. SLAC1 and KAT1) and transcription factors such as AREB/ABFs through their phosphorylation, causing stomatal closure and induction of ABA-responsive gene expression, respectively. In this study, to explore as-yet-unknown signaling pathways mediated by subclass III SnRK2s, we sought to identify subclass III SnRK2-interacting proteins. We mainly focused on SRK2D, because of its broad expression pattern in vegetative tissues and its important role in ABA signaling. We performed co-immunoprecipitation assays to isolate SRK2D complexes in planta and then employed LC-MS/MS to identify components of the SRK2D complexes. Interactions between subclass III SnRK2s and the candidate interactors were confirmed by Y2H and BiFC assays. A protein kinase named SDB1 was identified as a novel interactor of subclass III SnRK2s. Furthermore, SDR1, 2 and 3, which are closely related to SDB1, could interact with SRK2D. We will discuss physiological functions of SDB1, SDR1, 2 and 3 and their functional relevance with subclass III SnRK2s.

POS-TUE-229

THE *ARABIDOPSIS* NITRATE TRANSCEPTOR NRT1.1 GOVERNS DISTINCT SIGNALING PATHWAYS AND GOVERNS ROOT COLONIZATION VIA LOCAL MODIFICATION OF AUXIN FLUXES

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Nitrate (NO_3^-) is both the main nitrogen source for higher plants, and a signal molecule regulating their metabolism and development. The roots sense the NO_3^- concentration in the soil solution, and trigger signalling pathways allowing plant adaptation to its availability. Localized proliferation of lateral roots in NO_3^--rich patches is a striking example of the nutrient-induced plasticity of root development. On the basis of a fine RSA analysis, we showed that mutants of NRT1.1 displayed a strongly decreased root colonization, resulting from reduced lateral root elongation and delayed emergence. We then concluded that NRT1.1 acts as a NO_3^--sensor to detect local NO_3^- concentration. Further characterization of the underlying signalling mechanism showed that NRT1.1 acts not only as a NO_3^- transporter but also facilitates influx of the phytohormone auxin in a $NO3_3^-$ oncentration dependant manner. When external NO_3^- concentration is low, NRT1-1 transports auxin out of the lateral root primordium preventing its accumulation and thus lateral root development. This defines a new mechanism for sensing environmental stimuli, and for connecting nutrient and hormone signalling. Besides, NRT1.1 was also shown to regulate the expression of hundreds of genes in response to NO_3^- supply and Ho et al (2009) identified point mutations in the NRT1-1 protein that alter the NO3- transport activity but not the signalling function. The involvement of this signalling pathway and the effect of point mutations on the nitrate dependant root growth will be discussed.

POS-WED-230

THE ROLE OF SDG RECEPTOR-LIKE KINASES IN GERMINATION UNDER ABIOTIC STRESS

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Receptor-like kinases (RLKs), control a wide range of biological processes. The largest group of RLKs, with approximately 235 members in *Arabidopsis thaliana*, are leucine-rich repeat receptor-like kinases (LRR RLKs). Despite their large number, only a handful of plant LRR RLKs have been assigned a function, but it is clear that, as in humans, they are involved in key developmental pathways, as well as in hormonal signalling and defence responses. It is therefore of great importance to investigate this large group of proteins, to better understand how plants read and interact with their environment. We have been studying LRR RLKs involved in plant development under abiotic stress, and will present initial results obtained on a small family of “SDG” (for Salt Delayed Germination) proteins we identified for their role in germination under saline conditions. These results indicate a conditional, germination-specific phenotype, induced by both ionic and osmotic effects, and in which SDG members have specific but also partly overlapping roles.

POS-TUE-231

IN ARABIDOPSIS, THIOREDOXIN REDUCTASE TYPE C (NTRC) ORGANIZES ENHANCED THERMO-RESISTANCE BY ITS OXIDIZED AND REDUCED-DEPENDENT HOLDASE CHAPERON FUNCTION

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We had been indicated heat shock induction of transcripts for NADPH-thioredoxin reductase, type C (NTRC) by Genevestigator analysis in the light. Be discovered the overexpression of NTRC in *Arabidopsis* ($NTRC^{OE}$) resulting in enhanced resistance to heat shock, in other hands NTRC knock out mutant plant (ntrc1) exhibit a temperature sensitive phenotype. To investigate the underlying mechanism of this phenotype, we analyzed the protein’s biochemical characters and protein structure. NTRC assembles into homopolymeric structures of varying complexity with functions as a disulfide reductase, a foldase chaperone, and as a holdase chaperone. These multiple functions of NTRC are related with protein structure. High molecular weight(HMW) exhibited stronger activity as a holdase chaperone, whereas low molecular weight (LMW) showed weaker holdase chaperone activity but stronger disulfide reductase and foldase chaperone acitivities. Heat shock changed LMW proteins into HMW complexes. Mutations of the two active site Cys residues of NTRC into Ser (C217/454S-NTRC) caused a complete inactivation of its disulfide reductase and foldase chaperoene functions, but its holdase chaperone function decreased slightly. A degree of thermotolerance of the overexpression of the mutated C217/454S-NTRC assigned Arabidopsis was similar to that of $NTRC^{OE}$ plants. But $NTRC^{OE}$ plants, be treated longer incubation under heat stress, were stronger than C217/454S-$NTRC^{OE}$ plants to heat stress. These are suggest that thermotolerance of *Arabidopsis* was increased the heat shock-mediated holdase chaperone function of NTRC and the activity is significantly supported by NADPH.

POS-WED-232

FUNCTIONAL ANALYSIS OF B-CLASS HEAT SHOCK TRANSCRIPTION FACTORS IN ARABIDOPSIS

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Heat Shock transcription Factors (HSFs) are components of signal transduction in the heat stress response and are widely conserved among eukaryotes. In contrast to animals, which have only several HSFs, plants have evolved complicated HSF systems involving tens of members. Plant HSFs are classified into class A, B and C. B-class HSFs (HsfBs) are plant specific and have transcriptional repression domains. We investigated their functions in the heat stress response of Arabidopsis, which has 5 *HsfBs* among 21 *Hsfs* in total. HsfBs had the repressor activity and recognized Heat Shock Element (HSE) in a protoplast transient expression system. Thus we assumed that HsfBs act as transcriptional repressors and regulate heat stress responses by tuning or shutting down heat-inducible gene expression through HSE. To investigate the function of HsfBs in plant, we isolated *hsfb* single and double mutants and analyzed the expression patterns of heat-inducible genes under the heat stress conditions in these mutants. We found that the *hsfb2b hsfb4* double mutant exhibited stronger expression of several heat-inducible genes including *HsfA2* than the wild-type plant. Recently, it was reported that HsfB1 and HsfB2b are involved in the repression of heat-inducible genes. We compared the expression of heat-inducible genes between the *hsfb2b hsfb4* and *hsfb1 hsfb2b* double mutants.

POS-TUE-233

CONTROL MECHANISM OF OSMOTIC STRESS RESPONSE AND PLANT GROWTH BY POTASSIUM TRANSPORTER IN *ARABIDOPSIS*

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Osmotic adjustment plays a fundamental role in water stress responses and growth in plants. The potassium transporter KUP family plays important roles in this process under the control of ABA and auxin. We generated *Arabidopsis* multiple mutants for *KUP6*, *KUP8, KUP2/SHY3*, and an ABA-responsive potassium efflux channel, *GORK*. The triple mutants, *kup268* and *kup68gork*, exhibited enhanced cell expansion, suggesting that these KUPs negatively regulate turgor-dependent growth. The mutants showed increased auxin responses and decreased sensitivity to an auxin inhibitor (NPA) and ABA in lateral root growth. During water deficit stress, *kup68gork* highly impaired ABA-mediated stomatal closing. The *kup268* and kup68gork mutant plants decreased survivability to water deficit stress. An ABA-activated SNF1-related protein kinase, SRK2E, involved in a key component of ABA signaling, interacted with and phosphorylated KUP6. These results suggest that KUP functions are regulated directly via an ABA signaling complex. We propose that the KUP6 subfamily transporters act as key factors in osmotic adjustment by balancing potassium homeostasis in both cell growth and drought stress responses.

POS-WED-234

THE 1-CYS PEROXIREDOXIN FUNCTIONS NOT ONLY A REGULATOR OF SEED DORMANCY BUT A MOLECULAR CHAPERONE UNDER OXIDATIVE STRESS CONDITIONS

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Peroxiredoxins (Prxs) play critical roles in protection systems as peroxidases or molecular chaperones. The present study compares the molecular properties and biochemical functions of the three Prx isotypes (C1C-Prx, C2C-Prx, and C-PrxII) of Chinese cabbage, to gain insights into their concerted roles in plants. The three Prx isotype genes were dissimilarly expressed in tissue- and developmental stage-specific aspects. The transcript level of the C1C-Prx gene was teeming at the seed stage, but rapidly diminished after imbibitions. In contrast, the C2C-Prx transcript was not identified in the seeds, but its expression level increased at germination and was retained thereafter. The C-PrxII transcript level was slight at the seed stage, rapidly increased for 10 days after imbibitions, and gradually ceased thereafter. In the localization analysis using GFP-fusion proteins, the three isotypes showed different cellular distributions. In vitro thiol-dependent antioxidant assays revealed that the relative peroxidase activities of the isotypes were CPrxII > C2C-Prx > C1C-Prx. C1C-Prx and C2C-Prx, but not C-PrxII, prevented aggregation of malate dehydrogenase as a molecular chaperone. Taken together, these results indicate that the three isotypes of Prx play specific roles in the cells in timely and spatially different manners, but they also cooperate with each other to protect the plant.

POS-TUE-235

ERF115 GENE CODES FOR A TRANSCRIPTION FACTOR INVOLVED IN TOLERANCE TO HIGH SALINITY STRESS IN *ARABIDOPSIS THALIANA*

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Salinity of soils is one of the most important environmental abiotic stresses that impair agricultural productivity. Although many players involved in salt tolerance responses of plants have been described, there are still many components that remain unknown. Previously, we have identified *ERF115*, a gene coding for a putative transcription factor that belongs to the AP2 family protein. *ERF115* is selectively induced in roots by high salt treatments and the functional analysis of a homozygous mutant line showed to be less tolerant than the WT plants to salinity stress. Here, we show evidence indicating that this gene produces a protein that is localized in the nuclei of tobacco cells transiently transformed with pUBQ10::ERF115-Citrine fusion protein. Furthermore, we demonstrate that ERF115 is able to activate transcription, using a yeast one-hybrid assay. These results strongly suggest that ERF115 is a transcription factor involved in the salt stress response. To further inquire the role of this factor we performed a transcriptomic analysis of *erf115* mutant plants under control and salt stress conditions, using the Affimetrix arrays platform. In this analysis we identified 54 genes differentially expressed between *erf115* and WT plants; among them several genes involved in salinity stress response were found. These results indicate an important role for ERF115 in the tolerance to salinity stress, mainly by regulating target genes involved in the stress response.

POS-WED-236

AN EPIGENETICALLY-CONTROLLED TRANSCRIPTIONAL STATE UNDERPINS A PHYSIOLOGICAL ROLE FOR THE RELEASE OF HETEROCHROMATIC SILENCING UNDER HEAT

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Recently, it has been shown in A.thaliana that silent transgenes and some plant transposons and repetitive elements under epigenetic regulation at ambient temperatures, become transcriptionally activated throughout the whole plant by prolonged heat. The degree of activation is proportional to the time under stress, it does not become saturated before 30-48 hours of continuous heat, and it is accompanied by loss of nucleosomes and heterochromatin decondensation. In addition, chromatin assembly functions are required to restore silencing. Here, we show that a silent gene is released by long-term heat and fulfills a physiological role during the recovery phase from stress. Its transcriptional control is rather tissue-specific and depends on the stress-dose and temperature threshold, overall differing from the traditional heat-shock signaling pathways. The analysis of promoter-reporter constructs and epigenetic mutants (involved in the RNA-directed-DNA methylation pathway, histone modifications, DNA methylation and chromatin remodeling) imply a differential control of epigenetic regulation, with a potential mitotically-stable transcriptional state after heat-induction. Taking together, the observations support the notion that heat-induced disturbance of the chromatin landscape mediates a function under stress, acting as an apparent heat sensor. Variability of the induced transcriptional states across ecotypes of A. thaliana also suggests different points of regulation. We propose that the transcriptional-gene-silencing machinery was co-opted to the promoter area of this locus to fine-tune the expression under heat, thus generating a physiological role for the heat-induced release of heterochromatic silencing.

POS-TUE-237

FUNCTIONAL ANALYSIS OF NF-YC10, A NOVEL INTERACTING PROTEIN WITH *ARABIDOPSIS* DREB2A, WHICH MAY BE INVOLVED IN THE STRESS SPECIFIC EXPRESSION OF DREB2A TARGET GENES

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The *Arabidopsis* transcription factor DREB2A is one of the key regulators in water and heat stress responses. Under non-stress conditions, the DREB2A protein is degraded by the ubiquitin-proteasome system, but under dehydration- or heat-stress conditions, DREB2A accumulates and induces the expression of target genes. Previous studies have shown that the “negative regulatory domain (NRD)” composed of 30 amino acids contributes to the degradation of the DREB2A protein, and that the removal of the NRD significantly enhances the stability and activity of the DREB2A protein. Additionally, DREB2A specifically regulates the expression of dehydration or heat stress-inducible genes under each stress condition. Although it is assumed that DREB2A is post-translationally regulated, detailed mechanisms for the stabilization of DREB2A and the determination of stress specific expression patterns of its target genes remain to be elucidated. In this study, we identified NF-YC10, a member of the NF-YC family protein, as a novel interacting protein with DREB2A using yeast two-hybrid screening. NF-YC forms a heterotrimeric NF-Y transcription factor together with NF-YA and NF-YB. We found that transgenic *Arabidopsis* plants overexpressing *NF-YC10* showed enhanced resistance to heat stress but not to drought stress. Moreover, DREB2A-targeted heat-inducible genes were up-regulated under heat stress conditions in the *NF-YC10*-overexpressing plants. Now, we are analyzing gene expression patterns and stress resistance of *NF-YC10* T-DNA insertion mutants.

POS-WED-238

REGULATION OF NA^+ TRANSPORT, THE ROLE OF *ATHKT1;1* EXPRESSION IN COL-0 AND C24

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Salinity is a major threat to agriculture, resulting in reduced crop yields and endangering food security. With salt affected areas increasing, understanding the molecular mechanisms of salinity stress is of heightened importance. Elevated Na^+ levels lead to osmotic and ionic stress, both having significant effects on plant performance and yield. In particular, the accumulation of Na^+ in the shoot has been shown to be detrimental. The Na^+ transporter AtHKT1;1 is reported to be located in the xylem parenchyma of *A. thaliana* where it is involved in retrieving Na^+ from the xylem, thereby reducing the amount of Na^+ transported to the shoot. *A. thaliana* ecotype C24 was found to lack *AtHKT1;1* expression in the roots and accumulated significantly higher amounts of Na^+ in the shoot compared to ecotype Col-0. A tandem repeat 4 kb upstream of the start codon has been reported to enhance *AtHKT1;1* gene expression. We have observed significant polymorphisms within this region between C24 and Col-0 ecotypes. In addition, we have discovered significant sequence polymorphisms in other areas of the promoter and within the gene itself. Here we show that despite a disrupted CAAT motive, a highly polymorphic region 50 bp upstream of the start codon has only minor influence on the expression of *AtHKT1;1*. Furthermore, we investigate the role of a 1.6 kb insertion in the second intron of C24 on the expression of the gene.

POS-TUE-239

CHARACTERIZATION OF TRANSGENIC LINES CHANGED IN FLAVONOID BIOSYNTHESIS REVEALS CONTRIBUTION OF FLAVONOIDS TO FREEZING TOLERANCE IN *ARABIDOPSIS THALIANA*

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Flavonoids, such as flavonols and anthocyanins, are secondary plant metabolites with a variety of functions. It has been demonstrated that flavonoids accumulate in the cold and strong correlations were found between the content of different flavonols and leaf freezing tolerance in natural accessions of Arabidopsis. Furthermore, the expression of several genes encoding enzymes involved in flavonoid biosynthesis was higher in freezing tolerant than in sensitive accessions, also suggesting a role of flavonoid metabolism in freezing tolerance. However, these data provide no direct evidence for a function of flavonoids on plant freezing tolerance. We therefore analyzed the freezing tolerance (electrolyte leakage), the expression of flavonoid biosynthesis genes (qRT-PCR) and the content of secondary metabolites (LC-MS) of fifteen transgenic lines affected in different steps of the flavonoid biosynthetic pathway in comparison to the wild type under greenhouse conditions (non-acclimated control) and under cold acclimated conditions (two additional weeks of 4°C). A k.o. of specific genes affecting either the whole flavonoid biosynthetic pathway or only particular branches, lead to impaired freezing tolerance after cold acclimation, while overexpression of the gene encoding the transcription factor PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1) enhanced freezing tolerance through an increased accumulation of flavonols and anthocyanins. In addition, some null mutants without changes in freezing tolerance showed a massive accumulation of uncharacterized flavonoids that may functionally substitute well-characterized flavonoids that were no longer synthesized in these mutants. These data provide the first direct evidence for the contribution of flavonoids to plant freezing tolerance and cold acclimation.

POS-WED-240

COMPARATIVE ANALYSIS OF RICE AND WHEAT SEEDLING RESPONSES TO ANOXIA AND RE-OXYGENATION

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The model dicot and monocot plants, Arabidopsis and rice, display large differences in tolerance to anoxia. Arabidopsis rapidly dies when anoxic, while rice displays impressive growth and adaptation despite the energy crisis that occurs when oxygen is depleted. The peak of this tolerance is seen in the coleoptile, the only tissue to develop in rice seeds germinated under strict anaerobic conditions. Wheat is a dry-land winter crop and is intolerant to anoxia. We have studied changes in rice and wheat coleoptiles subjected to anoxia at the physiological, metabolite and protein level to better understand why plants differ so dramatically in their flooding tolerance. In contrast to wheat, rice was able to rapidly alter its proteome and metabolome during anoxia. Most interestingly, rice seems to accumulate many amino acids as well as the enzymes that synthesise them. This is in stark contrast to wheat and differs to what has been published on the Arabidopsis metabolome response to anoxia. Tolerance to re-oxygenation is a pre-requisite for when floodwaters recede, a consequence of which is oxidative stress. It is therefore puzzling that proteome changes in plants adapting to re-oxygenation is lacking in the literature. We will present preliminary results on proteome and metabolome changes that occur in re-aerated rice and wheat coleoptiles. Finally, we have analysed five wheat genotypes with purported differences in anoxia tolerance, showing surprising variation in growth resumption, metabolic profiles, cell damage and fermentation capacity.

POS-TUE-241

TRANSCRIPTOME ANALYSIS UNDER SALT STRESS IN *TRITICUM AESTIVUM*

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Environmental conditions including drought, salt, heat, and cold stress limit plant fertility of biomass and yield. Especially, relationships between resistance toward abiotic stress and gene expressions are typically controlled by complex interactions between genetically correlated traits. In addition, these relationships often result in tradeoffs in phenotypic expression. In this study, we elucidated gene expression profiles among several variety of bread wheat under salt stress condition. We tried to identify useful genes for salt stress tolerance in bread wheat, and will discuss about both the candidate genes and the future work on functional genetic studies of salt tolerance mechanism.

POS-WED-242

EFFECTS OF ELEVATED AMBIENT PRESSURE ON THE RATES OF DARK RESPIRATION AND NET PHOTOSYNTHESIS

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Effects of environmental stress on the net photosynthesis are investigated under artificially controlled environment. Especially, effects of elevated pressure on the net photosynthesis are investigated experimentally. This is because that the pressure is one of the most important factors for plant growth. It has possibilities of influence not only on the cells and chloroplasts in leaves directly but also on the CO_2 diffusion coefficient in leaves and concentration in apoplastic fluid. In this study, CO_2 exchange rate of aseptic medium cultured Arabidopsis thaliana was observed under the conditions of elevated ambient pressure using the pressure container made of acrylic and aluminium. Fluorescent lamps set up around the pressure container as the light sources. PPFD was controlled at 0 (dark) and 200 μmol m^{-2} s^{-1} (light), and ambient pressure at 0.1, 0.2 and 0.3 MPa. In addition, rates of dark respiration and net photosynthesis were measured by using an NDIR-gas analyser with a differential tube assembly. The results showed that the rate of net photosynthesis increases up to 1.2 times, whereas dark respiration rate also increased up to 1.1 times under increased pressure and proportionally increased partial pressure of CO_2. Additionally, the rate of net photosynthesis was reduced to one-third, whereas dark respiration rate also increased up to 3.5 times under increased pressure and constant CO_2 partial pressure. The results suggest unfound pressure stress responses through mechanical, biochemical, physiologic and genetic defences, and also a potential applicability to cultivate biomass more efficiently in the plant factory in future.

POS-TUE-243

GABA-GATED ANION CHANNELS IN PLANTS - THEY EXIST AND HAVE IMPORTANT PHYSIOLOGICAL ROLES

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Gamma-aminobutyric acid (GABA) is a neurotransmitter regulating membrane potential in nerve cells. GABA rapidly accumulates in plant tissues in response to various stresses, and regulates important processes such as pollen tube growth, root and hypocotyl elongation, and pathogen defence. There is much speculation that GABA signalling occurs in plant cells but definitive proof has been lacking, and the molecular components have remained elusive. We have identified GABA-binding sites within plant Aluminium-activated Malate Transporter (ALMT) proteins with homology to GABA-binding motifs of mammalian GABAA receptors (GABA-gated anion channels). Furthermore, we demonstrate that anion flux through ALMTs can be regulated by GABA and its analogs with an EC50 in the low micromolar range. Site-directed muatagenesis of this site alleviates GABA block but does not alter other channel properties. Using wheat ALMT1 we show that endogenous GABA, which accumulates in cells at low pH, closes the channel and inhibits cellular malate efflux. At higher pH ALMT1, which is expressed in root apical cells, is open and at alkaline extracellular pH functions to acidify the cell wall and maintain root growth. A survey of other ALMTs showed variable GABA sensitivity related to binding site residues. This may reflect different roles for GABA signalling in the context of the wide range of functions attributed to ALMTs. Our findings demonstrate that most ALMT proteins act as GABA-gated ion channels in plants that parallel the role of GABAA receptors in animals. This means GABA can be considered a conserved messenger for cellular communication across multiple kingdoms.

POS-WED-244

MOLECULAR MECHANISM OF TCP TRANSCRIPTION FACTOR IN RESPONSE TO ABIOTIC STRESS RESPONSE

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Drought is the major environmental threat to agricultural production and distribution worldwide. Plant adaptation to dehydration stress is a complex biological process that involves global changes in gene expression and metabolite composition. To discover novel master genes that regulate dehydration responsive pathway, we analyzed co-expressions of Arabidopsis genes using our dehydration-transcriptome data. We found 120 correlated-genes modules by co-expression analysis. In these modules, we analyzed gene-to-gene correlations of dehydration-repressed genes. Promoter analysis of these dehydration-repressed genes showed that Site II (GGNCCC) motifs were frequency found in their promoter regions. Site II is the predicted target sequence of TCP transcription factor. We characterized the Arabidopsis stress-inducible *TCP* genes and found that one of the TCP overexpressor (TCP-OX) showed dehydration and salt tolerance. The TCP-OX also showed narrow leaves under osmotic stress conditions. Microarray analysis showed that abiotic stress and ABA responsive genes were up-regulated in TCP-OX. These results suggest that TCP is important for balances of stress tolerance and leaf development under osmotic stress.

POS-TUE-245

MUTATION IN REPLICATION FACTOR C SUBUNIT 3 COMPROMISES PLANT REPAIR CAPABILITY TO REPLICATION DAMAGE IN *ARABIDOPSIS THALIANA*

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Abstract: Replication factor C (RFC) is an important protein complex that is involved in DNA replication and cell proliferation. It consists of one large subunit and four small subunits. The 3rd subunit of Arabidopsis RFC (*At*RFC3) is one of the 4 small subunits and a homologue of human RFC5. Our previous ethylmethane sulfonate screening identified that a G to A point mutation (in Gly) in *rfc3-1* led to enhanced induction of *PR* genes and resistance against *Hyaloperonospora arabidopsidis* (*H.a.*) Noco2. Here, the effects of *RFC3* mutation on resistance to toxicant MMS (Methyl Methanesulfonate) and cisplatinis were discussed. The suppressive effects on plant growth were found to be increased with the concentration of MMS and cisplatinis. However, the length of the root and the number of true leaves of *rfc3-1* is significantly smaller than that of wild type and *rfc3-1*/*RFC3* plants at 0.01 or 0.05 level, and the complementary plant *rfc3-1*/*RFC3* rescues the mutated phenotype of *rfc3-1*. Real time PCR analyses show that the expression of *GR1*, *KU70*, *MRE11*,*RAD51* and *BRCA1* is massive up-regulated with the treatment of 140 mg/L MMS or 40μmol/L cisplatin, and the primary as well the relative conductivity in *rfc3-1* plant is significantly higher than that of the wt and *rfc3-1*/*RFC3* plants. It is suggesting that mutation of *At*RFC3 compromises the resistance to DNA toxicant MMS and cisplatinis, and *At*RFC3 plays an important regulatory role in replication damage repairing in *Arabidopsis thaliana*.

POS-WED-246

TRANSLATING DROUGHT (TOLERANCE) RESEARCH FROM *ARABIDOPSIS* TO WHEAT

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In a study carried out in Arabidopsis, Pogson and colleagues discovered that mutations that disrupt the SAL1-PAP retrograde signaling pathway show enhanced drought tolerance. Loss of function mutations in the adenosine phosphatase, SAL1, enhances the capacity of plants to survive for up to 50% longer under drought (Wilson et al., 2009; Hirsch et al., 2011). The question is: can we translate this research in Arabidopsis to cereal crops and develop a drought tolerant wheat variety. In a first step to investigate the role of SAL1 in an Australian wheat variety, Chara, we identified seven SAL1 homeoforms in wheat and assigned their chromosomal locations to the A, B and D genomes. We PCR screened >15000 Heavy Ion Bombardment (HIB) mutant populations and identified 12 wheat lines harboring deletions in 4 out of 7 SAL1 homeoforms. We undertook a pairwise crossing project to combine these mutant SAL1 alleles in varying combinations. The drought tolerance, growth and yields of this germplasm will be characterized under both water stressed and non-stressed conditions in the glasshouse and field. The goal is to identify the best combination of SAL1 homeoform deletions that facilitate crops remaining greener, more turgid and photosynthetically active for longer periods in water deficit conditions.

POS-TUE-247

THE GLUTAMATE CARBOXYPEPTIDASE *AMP1* MEDIATES ABA AND ABIOTIC STRESS RESPONSES IN *ARABIDOPSIS*

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AMP1 (ALTERED MERISTEM PROGRAM1) encodes a glutamate carboxypeptidase that plays an important role in shoot apical meristem development and phytohormone homeostasis. We isolated a new mutant allele of *AMP1, amp1-20*, from a screen for abscisic acid (ABA) hypersensitive mutants, and characterized the function of *AMP1* in plant stress responses. *amp1* mutants displayed ABA hypersensitivity, while overexpression of *AMP1* caused ABA insensitivity. Moreover, endogenous ABA level was increased in *amp1-20* and decreased in *AMP1*-overexpressing plants under stress conditions. Application of ABA reduced the AMP1 protein level in plants. Interestingly, *amp1* mutants accumulated excess superoxide and displayed hypersensitivity to oxidative stress. The hypersensitivity of *amp1* to ABA and oxidative stress was partially rescued by reactive oxygen species (ROS) scavenging agent. Furthermore, *amp1* was tolerant to freezing and drought stress. The ABA hypersensitivity and freezing tolerance of *amp1* was dependent on ABA signaling. Moreover, *amp1* had elevated soluble sugar content and showed hypersensitivity to high concentrations of sugar. In contrast, the levels of amino acids were changed in *amp1* mutant compared to the wild type. This study suggests that AMP1 modulates ABA, oxidative and abiotic stress responses, and is involved in carbon and amino acid metabolism in *Arabidopsis*.

POS-WED-248

CLINAL VARIATION IN THE FREEZING TOLERANCE OF *ARABIDOPSIS THALIANA* ACCESSIONS AND MEMORY OF LOW TEMPERATURE PRIMING UNDER WARM CONDITIONS

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Plants native to temperate and cold climates increase their freezing tolerance during fall in preparation for winter frost. This natural priming response can be induced experimentally by exposing plants such as *Arabidopsis* to a short period of low temperatures. The large genetic and phenotypic diversity between natural accessions of *Arabidopsis* revealed evidence for geographical clines (both latitudinal and longitudinal) in acclimated and nonacclimated freezing tolerance, estimated from electrolyte leakage measurements on 54 accessions. Leaf proline (Pro) content was not correlated with freezing tolerance, while sugar content (Glc, Fru, Suc, Raf) was in the acclimated, but not the nonacclimated state. Expression levels of 14 cold induced genes were investigated before and after two weeks of cold acclimation by qRT-PCR. Expression of the *CBF1, 2* and *3* genes was not correlated with freezing tolerance. The expression of some CBF-regulated (*COR*) genes, however, was correlated specifically with acclimated freezing tolerance. A tight correlation between *CBF* and *COR* gene expression was only observed under nonacclimating conditions. During low temperature priming, gene expression and metabolism are strongly reprogrammed and plant freezing tolerance increases but very little is known about the persistence of low temperature priming under warm conditions, referred to as "de-acclimation". We therefore studied the kinetics of de-acclimation in selected accessions and analyzed gene expression by qRT-PCR, sugars, Pro and oxidative stress related responses. In addition, the memory of low temperature priming after de-acclimation was investigated after a low-temperature triggering response.

POS-TUE-249

CLASS IX ETHYLENE RESPONSE FACTOR (ERF) TRANSCRIPTION FACTORS INCLUDE MASTER REGULATORS OF ETHYLENE SIGNALLING AND PATHOGEN RESISTANCE

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Fungal diseases continue to cause major problems for agriculture worldwide. Translation of findings from model species such as Arabidopsis holds promise for addressing some of these industry issues. Our previous studies indicate that although it is transcriptionaly induced late upon pathogen infection, a class IX ERF transcription factor, *AtERF14*, is required for the ethylene and pathogen responsive expression of defence genes such as *PDF1.2*. Moreover, other class IX ERFs such as *ERF1* and *AtERF2* also required AtERF14 for ethylene or pathogen responsiveness, indicating AtERF14 performs a master regulator role in ethylene mediated defence responses. Consistent with this, *aterf14* mutants displayed enhanced susceptibility to the fungal pathogen, *Fusarium oxysporum*. Large scale gene expression analysis identified a discrete set of genes dependent on AtERF14 for pathogen-responsive expression. Among these were several Class IX ERFs, thereby confirming the master regulatory role of AtERF14. To study the conservation of ERF function beyond Arabidopsis, we identified *AtERF14* homologs in the model legume *Medicago truncatula*. A specific induction of class IX ERFs was associated with moderate resistance to the fungal pathogen *Rhizoctonia solani* and over-expression of an *AtERF14* homolog increased resistance to *R. solani* and *Phytophthora medicaginis*. Moreover, over-expression of class IX ERFs enhanced disease resistance without apparent impact on growth and development or symbiotic interactions with rhizobium in Medicago, suggesting enhanced resistance to root diseases can be uncoupled from symbiotic plant-microbe interactions in the same tissue.

POS-WED-250

ANALYSIS OF RESPONSES OF *ARABIDOPSIS THALIANA* TO INFECTION BY *ALTERNARIA BRASSICICOLA* USING METABOLITE PROFILING

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The ability of plants to withstand abiotic and biotic stress factors translates to their ability to survive and reproduce. A functional genomics platform was used to gain insights into the metabolic changes that occur following the infection of Arabidopsis with the necrotrophic fungal pathogen *Alternaria brassicicola*. We analyzed Arabidopsis mutants with known defects in defense signaling (dde2-2, ein2-1, sid2-2) along with wild-type Col-0. There were substantial changes in the level of metabolites following infection with *A. brassicicola*, with nearly 50% of the detected metabolites undergoing significant changes. Thus, there was a strong treatment effect but no significant genotype effects. For instance, ascorbic acid showed dramatic decrease following inoculation with *A. brassicicola*, but this pattern was the same across all the genotypes evaluated. Exogenous application of some of the metabolites showing significant treatment effects during *A. brassicicola* inoculation revealed that Gamma amino butyric acid (GABA) and xylitol promoted, while trehalose and ascorbic acid inhibited disease severity. Minimal media assays also revealed that GABA promoted the sporulation of *A. brassicicola* while ascorbic acid strongly inhibited fungal growth. Our data showed that ascorbic acid is important for resistance to this pathogen, and that metabolite profiling provides a robust approach to identifying compounds that limit pathogen growth and enhance plant resistance.

POS-TUE-251

ROOT MICROBIOME ASSEMBLAGE IS AFFECTED BY PLANT DEVELOPMENT THROUGH ROOT EXUDATION

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There is a concerted understanding of the ability of root exudates to influence the structure of rhizosphere microbial communities. However, our understanding of the connection between plant development, root exudation and microbiome assemblage is limited. Here, we analyzed the structure of the rhizospheric bacterial community associated with *Arabidopsis* at four time points corresponding to distinct stages of plant development: seedling, vegetative, bolting and flowering. Overall, there were no significant differences in bacterial community structure, but we observed that the microbial community at the seedling stage was distinct from the other developmental time points. At a closer level, phylum such as Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, and specific genera within those phyla followed distinct patterns associated with plant development and root exudation. These results suggested that the plant can select a subset of microbes at different stages of development presumably for specific functions. Accordingly, metatranscriptomics analysis of the rhizosphere microbiome revealed that 81 functional genes were significantly ($p<0.05$) expressed at different stages of plant development. For instance, genes involved in streptomycin synthesis were significantly induced at bolting and flowering stages presumably for disease suppression. In summary, the plant secretes blends of compounds and specific phytochemicals in the root exudates that are differentially produced at distinct stages of development to help orchestrate rhizosphere microbiome assemblage.

POS-WED-252

ERF 72 REGULATES FUSARIUM OXYSPORUM RESISTANCE IN ARABIDOPSIS

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Fusarium oxysporum is a root-infecting fungal pathogen that causes wilt disease on a broad range of plant species, including *Arabidopsis thaliana*. Investigation of the defense response against this pathogen has primarily been conducted using leaf tissues and little is known about the root defense response. In this study, we analysed the gene expression of Arabidopsis roots after infection with *F. oxysporum* by microarray analysis. In contrast to the leaf response, the root tissue did not show a strong induction of defense gene expression and instead showed a greater proportion of repressed genes. One of the genes repressed after infection was the transcription factor *ERF72* (ETHYLENE RESPONSE FACTOR72). ERF72 is a positive regulator of the JA pathway and a suppressor of programmed cell death. In addition, the *erf72* mutant showed increased resistance to *F. oxysporum* infection. This work explores the role of ERF72 in *F. oxysporum* resistance.

POS-TUE-253

RNASE L INHIBITOR PROTEINS PLAY A NEGATIVE ROLE IN PLANT RESPONSE TO OXIDATIVE STRESS AND BACTERIAL WILT

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RNase L inhibitor proteins (RLIs), an endogenous suppressor of RNA silencing, are highly conserved in eukaryotes and archaea. Their function in mammal defense against viruses has been shown; however, whether RLIs are involved in plant defense against biotic and abiotic stresses remains undetermined. Our study shows that the tomato RLI (SlRLI) shares highly significant sequence homology with orthologs in human, yeast and other plants. SlRLI-GFP protein is localized in mitochondria under normal condition. *SlRLI* is constitutively expressed in various tissues, and its expression is not significantly altered after treatments of various plant stress hormones and *Ralstonia solanacearum*, the causal agent of bacterial wilt (BW). Silencing of *RLI*s in tomato and *Nicotiana benthamiana* causes defective plant growth and increased tolerance to paraquat (MV). However, *RLI*-silenced tomato plants displayed reduced BW-tolerance, while *RLI*-silenced *N. benthamiana* conferred enhanced BW-tolerance. Characterization of Arabidopsis transgenic lines with reduced expression of *AtRLI2* or ectopic expression of *SlRLI* confirms the negative role of *RLI*s in plant defense against MV and BW. Consistently, the expression of genes involved in oxidative stress response, such as ascorbate peroxidases, is enhanced in plants with reduced *RLI* expression. These results together indicate that RLIs play a negative role in plant defense against oxidative stress and BW.

POS-WED-254

ACTIVATION OF R-MEDIATED INNATE IMMUNITY AND DISEASE SUSCEPTIBILITY IS AFFECTED BY DIFFERENT MUTATIONS IN A CYTOSOLIC O-ACETYLSERINE (THIOL) LYASE IN ARABIDOPSIS

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Cysteine synthases or *O*-Acetylserine (thiol) lyases (OASTLs) are evolutionary conserved proteins among many prokaryotes and eukaryotes that carry out sulfur acquisition and synthesis of cysteine. A mutation in the cytosolic OASTL-A1/ONSET OF LEAF DEATH3 (OLD3) was previously shown to reduce the OASTL activity of the old3-1 protein *in vitro* and cause autonecrosis in specific Arabidopsis accessions. Here we investigated why a mutation in this protein causes autonecrosis in some but not other accessions. The autonecrosis was found to depend on *Recognition of Peronospora Parasitica 1* (*RPP1*)-like disease resistance *R* gene(s), from an evolutionary divergent *R* gene cluster present in Ler-0 but not the reference accession Col-0. RPP1-like gene(s) show a negative epistatic interaction to the *old3-1* mutation which is not linked with reduced cysteine biosynthesis. Metabolic profiling and transcriptional analysis further indicates that an effector triggered-like immune response and metabolic disorder is associated with autonecrosis in *old3-1* mutants, likely activated by an *RPP1*-like gene. However, old3-1 itself renders largely neutral changes in primary plant metabolism, stress-defence and immune responses. Finally we showed that lack of functional OASTL-A1 results in enhanced disease susceptibility against infection with virulent and non-virulent *Pseudomonas syringae pv. tomato DC3000* strains. These results reveal an interaction between the cytosolic OASTL and components of plant immunity.

POS-TUE-255

GENETIC AND GENOMIC ANALYSIS OF *RHIZOCTONIA SOLANI* INTERACTIONS WITH ARABIDOPSIS

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Nectrotrophic fungal pathogens comprise some of the world's most important crop diseases. However, plant resistance mechanisms against necrotrophs are less understood than some of the other major types of pathogens. *Rhizoctonia solani* is an economically important soil-borne necrotrophic fungal pathogen, with a broad host range and little effective resistance in crop plants. Arabidopsis has been used as a model plant to study *R. solani*. (Perl-Travis et al., 2004; Foley et al., 2103). The Arabidopsis/ *R. solani* pathosystem we have developed exploits our findings that Arabidopsis is resistant to *R. solani* AG8 but susceptible to *R. solani* AG2-1. The Arabidopsis Affymetrix ATH1 Genome array was used to assess global gene expression changes in plants infected with AG8 and AG2-1 at seven days post-infection. While there was considerable overlap in the response, some gene families were differentially affected by AG8 or AG2-1 and included those involved in oxidative stress. Reverse genetic studies provided further evidence for a potential role in oxidative stress in resistance to AG8 in Arabidopsis. Perl-Treves R, Foley RC, Chen WQ, Singh KB (2004) Early induction of the Arabidopsis GSTF8 promoter by specific strains of the fungal pathogen Rhizoctonia solani. Molecular Plant-Microbe Interactions 17: 70-80. Foley, R.C, Gleason, G.A, Anderson, J.P, Hamann, T. and Singh. KB. (2013) Genetic and Genomic analysis of Rhizoctonia solani Interactions with Arabidopsis; Evidence of Resistance mediated through NADPH Oxidases. PLOS ONE. In press..

POS-WED-256

A CONSERVED SWEET SUCROSE TRANSPORTER IS KEY FOR NECTAR SECRETION IN EUDICOT FLOWERS

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The *abominable mystery* as put forth by Darwin and de Saporta links the evolution of both flowers and nectaries to the interactions between plants and insects and credits this coevolution for the rapid angiosperm diversification in the mid-Cretaceous. Nectar plays a key role in this interdependence of pollinating insects and flowers. Although nectar function and composition have been well characterized, the mechanism of nectar secretion remains unclear. Here, we identified a SWEET as the nectary-specific sucrose transporter in three Eudicot flowering plants: Arabidopsis, B. rapa and N. attenuata. We further show that this SWEET is essential for nectar production and acts as an efflux transporter. Understanding of the SWEET mechanism and phylogeny are two clues in elucidating the role of nectar in species diversification and in answering why self-pollinating plants retain nectar secretion.

POS-TUE-257

HYALOPERONOSPORA ARABIDOPSIDIS EFFECTORS MANIPULATE THE DEFENCE RESPONSE OF *ARABIDOPSIS THALIANA*

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The oomycete pathogen *Hyaloperonospora arabidopsidis* (*Hpa*) is the causal agent of downy mildew of *Arabidopsis thaliana*, a system that is used as a model for the study of plant-pathogen interactions. In order for successful colonisation, many biotrophic pathogens such as *Hpa* suppress and evade plant defences through secretion of effector proteins into the plant host to manipulate and disrupt the host immune system. Alignment of oomycete effector proteins has revealed a conserved amino acid sequence at the N-terminus with the consensus sequence RxLR (arginine, any, leucine, arginine), thus allowing the use of Bioinformatic approaches to identify putative effector proteins in the *Hpa* genome. Studying effector action and their targets in the host may help elucidate important components of the plant defence response, eventually leading to more durable crops. Here we present the interaction targets of an *Hpa* effector as determined by yeast two hybrid analysis. We have verified these interactions *in planta* and, using deletion and mutation analysis, identified the specificity of the interacting protein domains. We show that expression of the effector *in planta* alters host susceptibility to a range of pathogens and using microarrays have revealed effects on host transcription. Finally, work is being carried out to determine the biochemical function of the effector.

POS-WED-258

DEFENCE RESPONSES OF *ARABIDOPSIS THALIANA* TO INFECTION BY *BOTRYTIS CINEREA* ARE REGULATED BY THE CIRCADIAN CLOCK

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The circadian clock is an endogenous time-keeping mechanism that synchronizes biological processes with the external environment, so that they occur at optimal times of the day. While the clock has long been known to play a role in the anticipation of predictable daily changes in abiotic stimuli, several recent studies have implicated it in the anticipation of biotic interactions with as pathogens and herbivores. We are investigating whether the clock plays such a role in Arabidopsis in the immune response against the necrotrophic fungal pathogen *Botrytis cinerea*. Our results show that Arabidopsis has circadian clock-mediated variation in resistance to *B. cinerea*, with plants being least susceptible to infection in the subjective morning. In contrast, no such temporal variation in susceptibility was observed in arrhythmic *elf3* and *CCA1-ox* plants. Microarray analysis suggests that wild-type plants are able to mount a more rapid and robust defence response against *B. cinerea* when infected at subjective morning rather than in the evening. Significantly higher *B. cinerea* spore concentrations are required to cause disease symptoms in subjective morning versus evening infections suggesting that this clock-mediated regulation of the immune response can confer a fitness advantage to the host.

POS-TUE-259

CHARACTERIZATION OF ARABIDOPSIS CRT1 IN PLANT IMMUNITY AND GENOME STABILITY

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A genetic screen for components involved in resistance (R) protein-mediated immunity in Arabidopsis led to isolation of crt1 (compromised recognition of TCV). CRT1 was shown to be a MORC ATPase / endonuclease that physically interacts with multiple immune components. While CRT1 is mainly located in endosome-like vesicles in the cytoplasm, a subpopulation resides in the nucleus, which increases after infection. The combined findings that CRT1 i) is an endonuclease, ii) physically interacts with several components of the DNA repair and recombination (R/R) pathway, iii) is localized to heterochromatin, and iv) is implicated in epigenetic regulation, including suppression of heterochromatic transposable elements (TEs), suggest that CRT1 has an important nuclear function(s). Thus, we are investigating CRT1's role in the nucleus, particularly its involvement in stress-triggered genome stability, and to assess the importance of this function in plant immunity and evolution. To assess whether stress-triggered genome stability is regulated by CRT1, Southern blot analysis and second-generation sequencing are currently performed on consecutive generations of pathogen-inoculated wild type (WT) and mutant plants lacking CRT1 and its closest homolog CRH1. We are also testing whether trans-generational genomic instability facilitates development of novel alleles that enhance plant resistance to biotic stress. To investigate the function of nuclear CRT1, we altered CRT1 location (by fusion with a nuclear localization or nuclear exclusion signals) and monitored both disease resistance and the ability of these constructs to bind known DNA R/R proteins.

POS-WED-260

MEDIATOR: A NEW CONCEPT FOR PLANT DEFENSE REGULATION

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The Mediator multi-protein complex, first discovered in yeast by Nobel laureate Roger Kornberg, is conserved amongst all eukaryotes and plays an important role in regulating transcription. The function of the Mediator complex is to act as a "universal adaptor" between DNA-bound transcription factors and the RNA polymerase II complex. Our research has shown that manipulating genes encoding Mediator subunits offers yet unexplored control points to regulate disease resistance and flowering time in plants. We have found that the Mediator subunit, MED25 (previously known as the flowering regulator, PHYTOCHROME AND FLOWERING TIME1 (PFT1)) is required for the uncompromised expression of both salicylate- and jasmonate- (JA) associated defense genes and resistance and susceptibility to fungal and bacterial plant pathogens. We have recently shown that MED25 regulates JA-associated defense genes through interactions with the MYC2, MYC3 and MYC4 transcription factors, and the group IX sub-family of defense associated ERF transcription factors. We have since discovered additional Mediator subunits with roles in both defense and flowering time regulation. This work reveals a new layer of defense regulation previously undiscovered in plants and provides novel opportunities for agricultural improvement. (Kidd et al., 2009, The Plant Cell) (Cevik et al., 2012, Plant Physiology).

POS-TUE-261

AN ARABIDOPSIS CALMODULIN-LIKE PROTEIN ATCML13 CAN CONTROL THE FORMATION OF PEN1-SNAP33-VAMP722 TERNARY SNARE COMPLEX

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We previously identified for the first time in plants all three exocytotic component proteins (PEN1, SNAP33 and VAMP721/722) that comprise the ternary SNARE complex required for plant growth and immunity. However, PEN1 itself promiscuously forms the SNARE complex with multiple VAMPs in vitro, suggesting that an accessory protein may control the specific interactions of PEN1 with other SNAREs in vivo. We therefore isolated PEN1-interacting proteins by co-precipitation assays using GFP-PEN1-expressing transgenic plants, and identified AtCML13 by mass spectrometry. Although AtCML13 unlike typical calmodulins contains only three EF hands, it directly binds PEN1 in a calcium-dependent fashion. Binding of AtCML13 to the PEN1 linker region induces the formation of PEN1-SNAP33 binary t-SNARE complex as well as SDS-resistant PEN1-SNAP33-VAMP722 ternary SNARE complex. Since calcium is regarded as an important factor in plant disease resistance, our results suggest that AtCML13 may regulate the exocytotic immunity by controlling the formation of PEN1-SNAP33-VAMP721/722 SNARE complex.

POS-WED-262

UNDERSTANDING BENEFICIAL PLANT-ENDOPHYTE INTERACTIONS AT HIGH RESOLUTION

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P. indica is a recently discovered endophytic fungus that has the ability to colonize a wide range of hosts (including *Arabidopsis thaliana*). It has been shown to increase both biotic and abiotic stress tolerance and promote growth in a number of crop plants. While some of the host genes involved in the interaction between *Arabidopsis thaliana* and *Piriformospora indica* are known, a large fraction remain unidentified due to low-throughput technologies and low-resolution studies. I plan to use high-resolution studies in combination with next generation sequencing to elucidate cell-type specific transcriptional responses of *Arabidopsis* roots to colonization. I will report the results of these studies in my poster.

POS-TUE-263

INVESTIGATING THE LINK BETWEEN RNA PROCESSING, FLOWERING TIME AND DEFENCE

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Defense and development are often associated in animals. However, less is known about such associations in plants. Recently, we discovered a new example of this phenomenon which suggests that RNA processing, flowering time and plant defense are intimately linked in Arabidopsis. The Arabidopsis FPA gene encodes an RNA binding protein which modulates mRNA 3' end formation and delays flowering. *fpa* plants show increased resistance to the fungal pathogen *Fusarium oxysporum* (*Fo*) and are late flowering. FPA is a member of the 'autonomous pathway' of floral regulators, which promote flowering by repressing the floral repressor *FLOWERING LOCUS C* (*FLC*). We assessed the response of other loss-of-function 'autonomous pathway' mutants to *Fo* and identified four additional *Fo* susceptibility factors. To test the hypothesis that resistance to *Fo* in these mutants might be a pleiotrophic effect of delayed development, we rescued the late flowering phenotype of the *fpa* mutant by vernalisation and inoculated the vernalised plants with *Fo*. Resistance was retained after vernalisation, suggesting that resistance is conferred by a mechanism independent of flowering time. Resistance seems to be specific to *Fo* since the mutants did not respond differentially to the pathogens *Alternaria brassicicola* or *Pseudomonas syringae* in preliminary tests. To understand how these susceptibility factors affect the Fo response, current work focuses on hormone signaling pathways and defence gene expression in these mutants.

POS-WED-264

NON-SPECIFIC PHOSPHOLIPASE C2 IS INVOLVED IN DEFENCE RESPONSE OF *ARABIDOPSIS THALIANA* TO PSEUDOMONAS SYRINGAE ATTACK

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Members of large phospholipase family consisting of phospholipases A, D and phosphatidylinositol-specific phospholipase C (PLA, PLD, PI-PLC) were shown to play a role during responses of plants to pathogen attack. Non-specific phospholipase C family (NPCs) was discovered as a novel type of plant phospholipid-cleaving enzymes homologous to bacterial phosphatidylcholine-specific phospholipases C. Six genes (*NPC1-NPC6*) was described in *Arabidopsis* in 2005. Since then involvement of NPCs in responses to different environmental stresses was published. In our study, we found changes in *NPC* expression after leave inoculation with *Pseudomonas syringae*. Level of *NPC1*, *NPC2* and *NPC6* transcript was significantly reduced and level of *NPC4* and *NPC5* increased at 24 h after the treatment. Downregulation of *NPC2* expression was studied in details. It was rapid, detected already after one hour after the *P. syringae* treatment. Suppression of *NPC2* expression was triggered by both compatible and incompatible bacteria and by coronatin deficient mutant bacteria. *NPC2* expression was not suppressed by bacteria with impaired type III secretion system in longer time. Inoculation with elicitors flg22, elf18 and peptidoglycan caused decrease of *NPC2* transcript as well. Similarly, lower level of *NPC2* transcript was detected after salicylic acid treatment but not after methyljasmonate or ethylene treatment. The potential role of NPC2 in Arabidopsis defence reactions is discussed. This work was supported by Czech Science Foundation project no. P501/12/1942.

POS-TUE-265

OVEREXPRESSION OF THE DOF PROTEIN IN TOBACCO LEADS TO TRANSCRIPTIONAL ACTIVATION OF THE RESISTANCE GENE N, SUGGESTING POTENTIAL ROLES IN THE REGULATION OF HYPERSENSITIVE RESPONSE TO TMV

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Dof proteins are known as plant-specific transcription factors. We have been investigating the roles of the tobacco Dof family protein such as BBF1 in the defense response to *Tobacco mosaic virus* (TMV). Upon TMV infection, *N*-carrying tobacco cultivars such as Samsun NN induce the defense response called the hypersensitive response (HR). We cloned BBF1 ORF cDNA from tobacco and confirmed that a full-length recombinant BBF1 could bind *in vitro* to DNA with a Dof binding core motif found in the upstream regulatory region of the *N* gene. Using agrobacteria infiltration method, we found that the transient overexpression of BBF1 alone did not induce any HR but activated the endogenous *N* gene expression in Samsun NN. The *N* promoter activation by BBF1 overexpression was also confirmed in the *N*-lacking Samsun nn plant that was exogenously introduced an *N* regulatory sequence connected to a reporter gene. In addition, BBF1 overexpression enhanced not only ROS production but also expression of defense signaling marker genes as well as HR marker genes even without HR induction. Furthermore, the overexpression of BBF1 could stimulate the HR induction under the coexpression of the virus elicitor. Based on these data, we discuss potential roles of BBF1 as a transcription factor in the regulation of the defense responses including *N*-mediated HR induction.

POS-WED-266

CHLOROSIS CAUSING COMPOUNDS IN THE *ARABIDOPSIS-FUSARIUM* INTERACTION

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The culture filtrate of the hemi-biotrophic fungal plant pathogen *Fusarium oxysporum* has been shown to cause chlorotic lesions when applied to wild-type *Arabidopsis thaliana* leaf tissue. It was suspected that the fungus produced toxic metabolites in culture which detrimentally affect the plants tissue by inducing a senescence-like response similar to that of endogenously applied jasmonic acid (JA). We investigated whether the plants JA pathway was being induced by a JA mimic produced by our strain of *F. oxysporum*, but were unable to detect JA in the culture filtrate by liquid chromatography/mass spectrometry. However, JA-associated genes, specifically; CHI-B and VSP2, were induced in *Arabidopsis* leaves treated with the culture filtrate suggesting that compounds present in the filtrate did induced the JA pathway. Metabolomics analysis of the culture filtrate revealed a high number of organic and phenolic acids present in the filtrate. This correlated with the finding that the activity of the culture filtrate was pH dependent. The results from this study have provided a number of candidate compounds that could be responsible for the lesion phenotype induced by *F. oxysporum* culture. Further investigation into these metabolites will provide further understanding of plant defense signaling against *F. oxysporum* and potentially improve disease resistance in crop plants.

POS-TUE-267

CELL BIOLOGY OF ANTI-VIRAL SILENCING IN ARABIDOPSIS

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Plants use RNA silencing to regulate endogenes during development and environmental responses, and to protect against foreign nucleic acids including viruses and transposons. In Arabidopsis, post-transcriptional gene silencing (PTGS) is mediated by small interfering RNA (siRNA) and microRNA (miRNA) duplexes of 21-24bp, produced from double-stranded RNA (dsRNA) precursors by Dicer-like RNaseIII enzymes (DCL1-4). Selected si/miRNA strands are then loaded into Argonaute proteins (AGO1-10), which guide an RNA-induced silencing complex (RISC) based on sequence complementarity. Plant resistance to RNA viruses requires these same components, notably DCL2/4, AGO1/2, and the RNA-dependent RNA polymerase RDR6 and the RNA binding protein SGS3. While viral-derived small RNAs are clearly produced and loaded into AGO proteins, the molecular mechanisms underlying viral silencing are poorly understood. Here we report live-cell localization studies of silencing effectors during viral infection, and demonstrate recruitment of some effectors to the sites of viral replication.

POS-WED-268

DNA DEMETHYLATION AND DISEASE RESISTANCE

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An extensive screen for altered susceptibility to the necrotrophic fungus *Fusarium oxysporum* f.sp. *conglutinans* of various *Arabidopsis thaliana* mutants revealed that the triple mutant, deficient for the three major DNA demethylase enzymes *ros1*, *dml2* and *dml3* (*rdd*), showed increased susceptibility compared to WT. To understand the biology underlying this phenomenon, we performed microarray studies to determine difference in gene expression between WT and *rdd* mutant plants. We identified many genes with decreased expression levels, and the majority of them were found to be involved in disease or stress resistance. In-depth analysis of these genes revealed the presence of small repetitive elements (similar to small transposable elements) in the promoter regions of many of these genes. We asked whether these elements act as major regulators of gene expression in response to biotic stress, by altering gene expression levels via changes in DNA methylation. In fact, we show that the DNA methylation state of these repetitive elements is altered in WT plants, but not *rdd* mutant plants, upon *Fusarium oxysporum* infection. This difference in DNA methylation is reflected in altered gene expression of these genes. We hypothesise that these genes are targets of active DNA demethylation in response to biotic stress to achieve activation of gene expression.

POS-TUE-269

THE PAIRED R GENES RPS4 AND RRS1 FUNCTION TOGETHER IN EFFECTOR-TRIGGERED IMMUNITY

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Plant resistance (R) proteins have evolved to detect pathogen effector and activate defence. However, the molecular mechanisms leading to transcriptional reprogramming following recognition of effector by R protein remain mysterious. Arabidopsis *RPS4* (Resistance to Pseudomonas Syringae 4) and *RRS1* (Resistance to Ralstonia Solanacearum 1) form a resistance gene pair that activates defence after perception of either AvrRps4 or PopP2, two unrelated bacterial effectors. Both R proteins are TIR-NB-LRR and interestingly RRS1 carries a WRKY DNA binding domain, providing a potential link between pathogen perception and transcriptional changes. The *slh1* (sensitive to low humidity 1) mutant is a RRS1 allele that has lost WRKY DNA binding activity and shows lethal phenotype, which can be suppressed at high temperature or humidity. Transcriptomic analysis revealed a significant overlap between genes induced by temperature shift in *slh1* and by recognition of PopP2 in wild-type plants. Therefore, we generated an EMS-mutagenized *slh1* M2 population to identify suppressors of *slh1* immunity (*sushi*). We isolated 80 independent mutant families with various degree of phenotype suppression. Intriguingly, many of the *sushi* mutants with full reversion of phenotype carry non-synonymous mutations in the coding region of the *RPS4* gene. When transiently co-expressed, $RRS1^{SLH1}$ and RPS4 trigger cell death in *Nicotiana tabaccum*. RPS4-sushi mutations could fully suppress this cell death, as well as the one triggered by co-expression of RRS1, RPS4 and effector, demonstrating the functional requirement of RPS4 for RRS1 triggered immunity.

POS-WED-270

IDENTIFICATION AND CHARACTERIZATION OF AN ARABIDOPSIS MUTANT RESISTANT TO PHYTOPHTHORA INFECTION

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Oomycete pathogens cause serious damage to a wide spectrum of hosts, including many important crops and animals. They belong to the separate kingdom Stramenopila and *Phytophthora parasitica* is emerging as model oomycete species. We identified a T-DNA insertional *Arabidopsis thaliana* mutant resistant to *P. parasitica* and *P. capsici* infection. Pathogenicity assays of 9 additional T-DNA insertional mutants confirmed that *RTP1* (Resistant To *P. parasitica* 1) is responsible for resistance. Transformants of complementation and over-expression of *RTP1* were susceptible to *P. parasitica*, while *RTP1*-silenced transformants exhibited enhanced resistance. These results indicate that *RTP1* plays an important role in the compatible interaction between *A. thaliana* and *P. parasitica*. Cytological characterization showed that *rtp1* plants displayed localized cell death in response to infection by *P. parasitica*. *RTP1* encodes a membrane/endomembrane protein with multiple transmembrane domains as predicted by bioinformatic analysis. In further, particle bombardment mediated transient expression confirmed that RTP1-GFP is localized in the endoplasmic reticulum (ER). These results suggest that *RTP1* plays an important role in facilitating compatible *Arabidopsis-Phytophthora* interaction, maybe by negative regulation of ER-mediated cell death.

POS-TUE-271

A PUTATIVE SALICYLIC ACID TRANSPORTER EDS5 IS LOCALIZED TO THE PLANT CHLOROPLAST ENVELOPE MEMBRANE SYSTEM IN *ARABIDOPSIS*

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Salicylic acid (SA) is a phytohormone that plays a critical role in plant immunity. In response to pathogen infection, SA levels increase drastically within a few hours. SA accumulation leads to activation of a set of SA-dependent defense responses, including differential expression of defense genes, such as pathogen-related (PR) genes and increases in the levels of phenolics and phytoalexines, which limit the growth of virulent pathogens in susceptible plants. Thus, SA is a central signaling molecule controlling plant innate immunity. Chloroplasts are responsible for biosynthesis of SA an important signal molecule in plant immunity. EDS5 is a homolog of the MATE (multidrug and toxic compound extrusion) family of transporters, and is essential for SA biosynthesis. It has been speculated that EDS5 would be involved in the export of SA from chloroplasts. However, the subcellular localization of EDS5 remains largely uncharacterized. We demonstrate here that EDS5 is specifically localized to the chloroplast envelope membrane in *Arabidopsis*. In addition, we found that EDS5 is preferentially expressed in epidermal cells. These findings suggest that EDS5 is responsible for transport of SA from chloroplasts to the cytoplasm in epidermal cells.

POS-WED-272

CONTROL OF DEFENSE GENE EXPRESSION VIA CHLOROPLAST CA2+ SENSOR PROTEIN CAS IN *ARABIDOPSIS THALIANA*

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In addition to photosynthesis, the chloroplasts are responsible for the biosynthesis of stress hormones, such as salicylic acid (SA) and jasmonic acid (JA); and the generation of signal molecules, such as ROS and NO. However, the role of chloroplasts in plant immunity remains largely uncharacterized. Previously, we found that, the pathogen elicitor signals are quickly transmitted to chloroplasts and evoke a transient increases in stromal Ca^{2+} concentration. A thylakoid localized Ca^{2+} sensor protein CAS is responsible for elicitor-induced SA biosynthesis and subsequent defense responses. Microarray analysis demonstrated that 827 genes were down-regulated and 403 genes were up-regulated in elicitor (flg22)-treated CAS deficient mutant *cas-1*. Gene ontology analysis revealed that CAS is responsible for PAMP-induced expression of defense genes and suppression of chloroplast gene expression. We identified many CAS-dependent transcription factors, including WRKYs, suggesting a crucial role of WRKYs in CAS-dependent defense gene expression. One third of the CAS-dependent genes overlapped with chloroplast-derived $^1O_2$ responsive genes, suggesting the possibility that chloroplast-derived $^1O_2$ might act as a retrograde signal to activate expression of defense genes. Interestingly, it has been shown that CAS is phosphorylated in a Ca^{2+} dependent manner, and might be involved in the cyclic electron flow at PSI. PGRL1A and PGRL1B are thylakoid-localized proteins, which are involved in the regulation of the cyclic electron flow. We demonstrated that CAS and PGRL1A interact with each other in vivo, suggesting a possible role of CAS in the modulation of cyclic electron flow. We will present a novel model for the chloroplast control over plant immunity through CAS.

POS-TUE-273

VIRAL SMALL INTERFERING RNAS TARGET HOST GENES TO MEDIATE DISEASE SYMPTOMS IN PLANTS

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The *Cucumber mosaic virus* (CMV) Y-satellite RNA (Y-Sat) has a small non-protein-coding RNA genome that induces yellowing symptoms in infected *Nicotiana tabacum* (tobacco). How this RNA pathogen induces such symptoms has been a long standing question. We show that the yellowing symptoms are a result of small interfering RNA (siRNA)-directed RNA silencing of the chlorophyll biosynthetic gene, CHLI. The CHLI mRNA contains a 22-nucleotide (nt) complementary sequence to the Y-Sat genome, and in Y-Sat-infected plants, CHLI expression is dramatically down-regulated. Small RNA sequencing and 5-Prime RACE analyses confirmed that this 22-nt sequence was targeted for mRNA cleavage by Y-Sat-derived siRNAs. Transformation of tobacco with a RNA interference (RNAi) vector targeting CHLI induced Y-Sat-like symptoms. In addition, the symptoms of Y-Sat infection can be completely prevented by transforming tobacco with a silencing-resistant variant of the CHLI gene. These results suggest that siRNA-directed silencing of CHLI is solely responsible for the Y-Sat-induced symptoms. Furthermore, we demonstrate that two *Nicotiana* species, which do not develop yellowing symptoms upon Y-Sat infection, contain a single nucleotide polymorphism within the siRNA-targeted CHLI sequence. This suggests that the previously observed species specificity of Y-Sat-induced symptoms is due to natural sequence variation in the CHLI gene, preventing CHLI silencing in species with a mismatch to the Y-Sat siRNA. Taken together, these findings provide the first demonstration of small RNA-mediated viral disease symptom production and offer an explanation of the species specificity of the viral disease.

POS-WED-274

HAT4, A NOVEL *ARABIDOPSIS THALIANA* MICRORNA RESPONSIVE TO *PHYTOPHTHORA* INFECTION

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Small noncoding RNAs contribute significantly to the plant innate immunity system in bacteria. Oomycetes are fungus-like microbes causing severe damage to the agriculture, forestry and the entire ecosystems. However, the roles of small RNAs in plant-oomycete interaction are largely unknown. We employed the developed *Arabidopsis*-oomycete pathosystem and cloned a novel *Arabidopsis* microRNA (HAT4) that specifically accumulated during *Phytophthora parasitica* and *P. capasici* infections, but not induced by biotic stress of fungal and bacterial infections, nor by the abiotic stresses, including osmotic and hormonal stresses induced with NaCl, mannitol and ABA, respectively. HAT4 is shown to be associated with host susceptibility: first, the increased accumulation of HAT4 is coincided with the disease progression; and second, the accumulation of HAT4 is positively correlated with the level of pathogen colonization. HAT4 expression is accompanied with the success of *Phytophthora* colonization, being induced by both root and leaf inoculation with zoospores or mycelia. Preliminary transient expression of *GUS* fusion gene showed that HAT4 promoter was likely to be manipulated by pathogen factors that are conserved and specifically expressed in *Phytophthora* and are capable of entering host nuclei. Bioinformatic analysis suggested that HAT4 is likely to target a transcription factor. Thus HAT4 is a central noncoding small RNA important for host immunity against oomycete pathogens.

POS-TUE-275

THE CRYSTAL STRUCTURE OF THE HETERODIMER FORMED BETWEEN THE TOLL-INTERLEUKIN 1 RECEPTOR (TIR) DOMAINS OF THE ARABIDOPSIS R PROTEINS RRS1 AND RPS4

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A plant's ability to detect and resist the infection of a specific pathogen rests with two critical genes; a resistance (R) gene in the plant and a corresponding avirulence (effector) gene in the pathogen. The proteins products of R gene's play a surveillance role within the plant cell and stimulate defence signalling after recognition of a specific effector protein. The most predominant class of R genes encode tridomain proteins with a central nucleotide-binding (NB) domain, a C-terminal leucine rich repeat (LRR) and either a Toll-interleukin 1 receptor (TIR) domain or a coiled-coil (CC) domain at their N-terminus. Interesting it appears that for resistance to some pathogen isolates two NB-LRRs are required. An explicit example of this is presented in Arabidopsis whereby the TIR-NB-LRR proteins RPS4 and RRS1 are both required for resistance to three different pathogens. We have demonstrated that the TIR domain of RPS4 and RRS1 can form a direct and specific interaction, in vitro, implicating a role for the TIR domains in coordinating dual resistance. In addition, we report the crystal structure of the TIR domain of both RRS1 and RPS4, individually and in a heterodimer complex. The heterodimer structure reveals the interface that mediates the interaction between RPS4 and RRS1 TIR domains. We are currently investigating mutations that disrupt this interaction and surveying any functional affects in an effort to understand further the molecular basis of R protein mediated resistance signalling.

POS-WED-276

SHIFT OF NPR1 OLIGOMERIC STATUS MEDIATED BY NITRIC OXIDE REGULATES BASAL DISEASE RESISTANCE

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Nitric oxide (NO) is emerging as a key signalling molecule in both plant development and immunity. Arabidopsis *nox1*, an NO overproducing mutant, has been reported to have diverse developmental phenotypes. However, there is no report on the possible impact of *nox1* on disease resistance. Our findings suggest that *nox1* exhibit increased NO production, decreased salicylic acid (SA) accumulation, attenuated *PR1* expression and compromised basal disease resistance against *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000. In addition, we have generated and characterised *nox1 S-nitrosoglutathione reductase 1* (*atgsnor1*) double mutants. Mutations in *atgsnor1* modulate total cellular levels of S-nitrosylation, the addition of NO moiety to a cysteine (Cys) residue to form an S-nitrosothiol (SNO). Therefore, these mutant lines may uncover distinct roles for NO and SNOs. While *nox1* plants show less susceptibility than *atgsnor1-3* in response to DC3000, the *nox1 atgsnor1-3* double mutant shows significantly enhanced disease susceptibility compared to either the *nox1* or *atgsnor1-3* single mutant. This result suggests that both NO and GSNO function additively to enhance pathogen susceptibility, with a dominant role for GSNO. Furthermore, the *nox1* mutant shows significantly less NPR1 monomerisation shift from the oligomer status in response to SA treatment compared to wild type, suggesting the enhanced susceptibility of *nox1* might be associated, at least in part, with decreased NPR1 monomerisation. BY was funded by BBSRC grant BB/D0118091/1 to the Loake lab. This work was supported by a grant from the Next-Generation BioGreen 21 Program (SSAC, grant# : PJ009011), Rural Development Administration, Republic of Korea.

POS-TUE-277

A PUBLIC METABOLOME DATABASE TOOL ENABLES RAPID CLASSIFICATION OF ARABIDOPSIS PHOTORESPIRATORY MUTANTS VIA METABOLITE PROFILING

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Most enzymes comprising the core photorespiratory cycle of *Arabidopsis thaliana* and the genes encoding them are well known. However, the hunt for genes involved in photorespiration is far from over. New forward genetic screens for *A. thaliana* mutants with altered photorespiratory function - such as those based on high-throughput chlorophyll fluorescence assays – show promise for the isolation of novel photorespiratory mutants. However, inexpensive methods to rapidly discriminate novel mutants from mutants affected in well-known photorespiratory genes are required to avoid the wasting of time and resources re-characterising known mutants. To this end, we present here a public database of reference metabolic phenotypes recorded for a variety of well-known *A. thaliana* photorespiratory mutants together with a simple statistical web tool - the *MetabolomeExpress PhenoMeter* - enabling researchers to classify photorespiratory mutants on the basis of similarity of metabolic phenotypes to known phenotypes in the reference database. To demonstrate the effectiveness of the tool, we used it to classify a set of *A. thaliana* EMS mutants isolated from a recent chlorophyll fluorescence-based forward genetic screen before re-sequencing their genomes to check for mutations in known photorespiratory genes. This approach enabled the rapid and cost-effective discrimination and functional classification of novel photorespiratory mutants displaying metabolic signatures of photorespiratory dysfunction without genetic lesions in any known photorespiratory genes.

POS-WED-278

FUNCTIONAL CHARACTERIZATION OF XYLOSE ISOMERASE FROM ARABIDOPSIS

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The plant cell has a range of biochemical mechanisms to deal with free sugars generated during cell wall restructuring, from storage carbohydrates, as a result of microbial infections and as the result of glycoprotein turnover to name a few. A range of monosaccharides including galactose, arabinose, galacturonic acid, fucose and glucuronic acid can be recycled into nucleotide sugars through characterized salvage pathways. Xylan is the second most abundant polysaccharide in the plant cell wall containing a high proportion of the monosaccharide xylose. In plants, it is currently unclear whether xylose is salvaged into the nucleotide sugar UDP-Xyl or whether it is remobilized into carbon metabolism via the pentose phosphate pathway. Recently we identified the initial step in the xylose remobilization pathway (xylose isomerase) in Golgi enriched fractions of Arabidopsis. Xylose isomerase (XI) which catalyses the isomerisation of xylose to xylulose has been functionally characterized in bacteria, fungi and indirectly in plants nearly two decades ago. Given the enzymes association with Golgi membranes, we sought to re-examine the function and role of this enzyme in plants. Only one xylose isomerase (AT5G57655) has been computationally assigned in Arabidopsis but no direct biological analysis has been reported. Here we ectopically expressed the Arabidopsis xylose isomerase in baking yeast (*Saccharomyces cerevisiae*) to confirm its biological function. The xylose isomerase expressing yeast are capable of using xylose as the carbon source and confirms their biochemical function as xylose isomerases. Transiently expressed xylose isomerase fused to YFP in onion epidermal cells showed that the xylose isomerase protein is membrane associated. Promoter-GUS expression constructs are currently being analyzed, while virus induced gene silencing in tobacco plants is being used to determine the effect of gene silencing on this pathway.

POS-TUE-279

ECH2 AND H^+-PYROPHOSPHATASE CORRELATIVELY ACT IN OILSEED MOBILIZATION DURING GERMINATION

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Compensation, whereby reduction of cell proliferation below a threshold-level triggers enhanced post-mitotic cell expansion in leaf primordia, implies an interaction between these cellular processes during organogenesis and provides clues to understand organ-size regulation. We have tried to reveal the mechanisms behind the compensation, by using mutants and transgenics of Arabidospsis. In Arabidopsis, conversion of seed storage lipids into carbohydrates is essential for post-germinative heterotrophic growth. We recently discovered that in the *fugu5*, the loss-of-function mutant of the vacuolar H^+-pyrophosphatase (H^+-PPase), gluconeogenesis is compromised due to the high cytoslic levels of pyrophosphate (PPi). Therefore, it appears that PPi inhibits several cellular functions, including cell cycling, whereby it seems to trigger compensated cell enlargement (CCE). Here, in order to understand the genetic pathways involved in CCE, we mutagenized *fugu5* seeds by $^{12}C^{6+}$ heavy-ion irradiation, and conducted a screening of mutations that negatively affect CCE. Interestingly, we isolated A-3-1 mutant line in which cell size was severely reduced but the cell number was similar to *fugu5* of origin. Importantly, in the A-3-1 single mutant, cell number remained unaffected, but cell size was almost equal to that of the wild type. Surprisingly, A-3-1 mutation did not affect at all CCE in other compensation exhibiting mutant backgrounds, *an3-4* and *fugu2*. The gene mutated in A-3-1 line was the *enoyl-CoA hydratase 2* (*ECH2*). The correlative function of ECH2 and H^+-PPase in storage lipid mobilization and its impact on cell expansion will be discussed.

POS-WED-280

ROLE OF THE CIRCADIAN CLOCK IN COORDINATION OF GROWTH WITH PRIMARY METABOLISM

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The circadian clock enables organisms to time responses to rhythmic environmental changes. Optimal growth is attained when the circadian rhythm matches environmental cycles; it has been proposed that this is due to the need to correctly time starch turnover to the 24 h cycle. Plants buffer themselves against the daily alternation of light and dark by accumulating photosynthate as starch in the light, and remobilizing it to support metabolism and growth at night. They adjust to short days by increasing the rate of starch synthesis and decreasing the rate of starch degradation, such that starch is not entirely exhausted at dawn. This maximizes growth by ensuring that newly fixed carbon is immediately invested in growth while avoiding periods of starvation before dawn. To explore the link between the clock, metabolism, growth we carried out two experiments in the EU TiMet project. Wild-types growing in five photoperiods were harvested at 2h intervals and analysed for clock transcripts, starch, metabolites, enzyme activities, photosynthesis, respiration, polysome loading and growth. Shorter photoperiods lead to a shift in the phasing of clock gene and PIF transcripts. Quantitative modeling of the measured dataset uncovered a close coordination of starch turnover with night growth even in the shortest photoperiods. We propose a mechanism that stimulates starch synthesis in short photoperiods. Furthermore, a set of core clock mutants were grown in a light-dark cycle and harvested at 2h intervals in their growth conditions, as well as after transfer to continuous light. The results uncover multiple impacts of the clock on central metabolism, affecting not only starch but also N metabolism, and point to reasons for these changes.

POS-TUE-281

SUCCINATE DEHYDROGENASE ASSEMBLY FACTOR 2 IS NEEDED FOR ASSEMBLY AND ACTIVITY OF MITOCHONDRIAL COMPLEX II AND FOR NORMAL ROOT ELONGATION IN ARABIDOPSIS

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Succinate dehydrogenase (SDH) plays a central role in respiratory metabolism as a component of both the electron transport chain and TCA cycle. We report the identification of an SDH assembly factor by analysis of T-DNA insertions in At5g51040, a protein with unknown function identified by mass spectrometry analysis. This gene is co-expressed with a number of genes encoding mitochondrial proteins, including SDH1-1, and has low partial sequence similarity to human SDHAF2, a protein required for flavin-adenine dinucleotide (FAD) insertion into SDH. In contrast to observations of other SDH deficient lines in Arabidopsis, the sdhaf2 line did not affect photosynthetic rate or stomatal conductance, but instead showed inhibition of primary root elongation with early lateral root emergence, presumably due to the low SDH activity caused by the reduced abundance of SDHAF2. Both roots and leaves showed succinate accumulation but different responses in the abundance of other organic acids and amino acids assayed. Isolated mitochondria showed lowered SDH1 protein abundance, lowered maximal SDH activity and less protein-bound FAD at the molecular mass of SDH1 in the gel separation. The short root phenotype and SDH function of sdhaf2 was fully complemented by transformation with SDHAF2. Application of the SDH inhibitor, malonate, phenocopied the sdhaf2 root architecture in WT. Whole root respiratory assays showed no difference between WT and sdhaf2, but micro-respirometry of the tips of roots clearly showed low oxygen consumption in sdhaf2 which could explain a metabolic deficit responsible for root tip growth.

POS-WED-282

THE 'FUNGICIDE' PHOSPHITE AND ITS EFFECTS ON PLANT METABOLISM AND GENE EXPRESSION

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Phosphite (Phi, $H_2PO_3^-$) is the active component in phosphonate-based fungicides that are widely used to combat plant pathogens, e.g., (hemi-) biotrophs such as oomycetes of the genus Phytophthora. Phi-induced pathogen resistance is well established; however, its molecular basis is unknown. The structural similarity of Phi to the plant macronutrient phosphate (P_i, $H_2PO_4^-$) has also sparked investigations into its effects on plant phosphorus (P) signalling networks. In this study, we report a detailed physiological and molecular characterisation of Phi effects on P-limited plants. Our results suggest that Phi is not a generic mimetic of P_i, but rather interacts with distinct components of plant nutrient signalling networks: while it attenuates anthocyanin accumulation in P-limited leaves and reduces both root hair length and density to a similar extent as P_i, it also leads to a much stronger arrest of primary and secondary root elongation as well as a more general suppression of plant growth. Despite these pronounced effects on the plant's morphology and physiology, Phi is unable to reduce the high transcript levels of most P-starvation induced genes in the first 8 to 24 h, which is in stark contrast to P_i resupply. At the same time, metabolite profiling reveals tissue-specific early perturbations in sulfur (methionine, glutathione) and nitrogen (nitrate, serine, threonine, arginine) assimilation as well as lipid metabolism (sulfo-, phospholipids, triacylglyerols), which were confirmed by corresponding changes in transcript profiles. Therefore, we conclude that the effects of Phi only partially overlap with plant responses to changes in phosphorus status and have much wider implications for reprogramming of plant metabolism that can ultimately lead to an increased alertness to the presence of pathogens.

POS-TUE-283

A NOVEL VACUOLAR SUGAR CARRIER IS INVOLVED IN CELLULAR SUGAR HOMEOSTASIS IN ARABIDOPSIS

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During the life cycle of a plant, sugars fulfil a remarkable number of important functions. They not only form the basis of energy for metabolic processes, but also serve as a precursor in the synthesis of starch and cellulose, as well as in the synthesis of carboxylic- and amino acids. The large central vacuole of mesophyll and storage cells, which currently is best characterized in terms of its function, serves as storage for these primary metabolites. Many of these substances are transported through the tonoplast via carriers of which the general biochemical characteristics are already known, but to date only a few carriers have been identified on the molecular level.By the Use of different physiological approaches, we have identified a novel vacuolar sugar carrier, which takes part in the sugar homeostasis between the vacuole and the cytosol.

POS-WED-284

MODULATION OF PLANT COMPOSITION BY AN ORPHAN GENE OF ARABIDOPSIS

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Deficiency in dietary protein is globally one of the most severe health problems, acutely affecting over 50 million children. The *QQS* gene of Arabidopsis modulates starch composition but is not recognizable by sequence homology in other species (Li et al., 2009); transgenic lines with up- or down-regulated *QQS* expression have a normal appearance but an altered starch content. We hypothesized QQS may be conserved in a feature other than primary sequence, and as such could function to regulate composition in another species. To test the potential of *QQS* in affecting composition in a major crop plant, we introduced *QQS* into soybean and maize. Soybean expressing *QQS* have indistinguishable morphology from controls, but 5-80% decreased leaf starch and 6-60% increased leaf protein; seeds contain 0-13% less oil and protein content is increased by 10-18%. These data broaden the concept of *QQS* as a modulator of carbon allocation, and demonstrate that this species-specific gene can affect the composition of seeds of an agronomic crop thought to have diverged from Arabidopsis over 100 million years ago. Maize plants expressing QQS have similar morphology to the controls, with increased protein, similar oil and a-bit-decreased starch in kernels. *QQS* is among the approximately 5-20% of gene models in eukaryotic genomes that encode proteins that lack sequence homology with any known motifs (POFs, proteins with obscure features) and also are species-specific (an orphan gene). Thus, *QQS* can affect composition in non-native species. Taken together, the data indicate *QQS* as a novel regulator of plant composition, and begin to reveal the skeleton of a previously undefined network in which *QQS* participates.

POS-TUE-285

AN EXAMINATION OF THE ENZYMATIC PROPERTIES OF A BROAD FAMILY OF ALANINE AMINOTRANSFERASES

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Alanine aminotransferase (AlaAT) has been studied in a variety of organisms due to the involvement of this enzyme in mammalian processes such as non-alcoholic hepatocellular damage, and in plant processes such as C4 photosynthesis, post-hypoxic stress response and nitrogen use efficiency (NUE). Previous studies have shown that over-expression of barley AlaAT in crop plants, such as canola and rice, results in an NUE phenotype however, the underlying reasons for these phenotypic observations are not understood. To better understand the increased NUE of crop plants over-expressing AlaAT, analysis of the enzymatic properties of a wide variety of AlaAT enzymes was conducted in bacteria, along with a phenotypic characterization of Arabidopsis thaliana plants expressing various AlaAT enzymes. We present a direct kinetic comparison of glutamic:pyruvic transaminase (GPT) activity for seven AlaATs and two glutamate:glyoxylate transaminases (GGT), measuring the KM values for the enzymes analyzed. We also demonstrate that alterations in nitrogen source and concentration confer significant phenotypic differences in root length and rate of plant growth in AlaAT over-expressing plants, along with alterations in protoplast uptake of amino acids. The AlaAT enzyme differences identified here indicate that AlaAT homologues have differentiated significantly and the roles these homologues play in vivo may also have diverged significantly. Specifically, the differing kinetics of AlaAT enzymes and how this may alter the nitrogen use efficiency in plants, including model plants and cereals, will be discussed.

POS-WED-286

MITOCHONDRIAL TRANSLATION EFFICIENCY MEDIATED BY LETM PROTEINS LEADS TO ENHANCED DROUGHT STRESS TOLERANCE IN *ARABIDOPSIS THALIANA*

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Mitochondria are considered as essential “powerhouses”, respiring carbon and providing energy and redox equivalents within the cell. Perhaps counterintuitive to this concept, studies in animals have shown that inhibition of mitochondrial biogenesis and translation leads to increased cell longevity. In plants, the phenomenon of decreasing global mitochondria function and its consequence(s) has received far less attention. Here we characterised two nucleus-encoded, mitochondrial-localised LEUCINE ZIPPER-EF-HAND-CONTAINING TRANSMEMBRANE (LETM) proteins required for efficient translation of mitochondrial encoded transcripts in Arabidopsis. A decrease in both isoforms of LETM leads to a marked reduction in Complex V steady state levels. Consequently, a significant decrease in ATP levels was observed. Metabolic profiling also revealed enhanced starch and ascorbate levels and a decrease in reactive oxygen species (ROS) generation. Testing abiotic stress tolerance in letm1(-/-) LETM2(+/-) double mutants showed an enhanced tolerance to water deficit and a faster recovery response upon rehydration. Transcriptomic and metabolomic profiling performed subsequently provided novel insight into the molecular signaling mechanisms involved in this enhanced drought response, mediated as a result of cell wide shifts in redox control. Taken together, this study provides novel insights to global mitochondrial efficiency, carbon partitioning and redox control upon drought stress.

POS-TUE-287

INVESTIGATION OF THE FLAVIN COFACTOR METABOLISM USING *ARABIDOPSIS* AS A MODEL SYSTEM

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FMN and FAD are important cofactors for a variety of enzymes involved in a multitude of metabolic processes in all organisms. These cofactors, as well as their inactive precursor riboflavin, are known to be interconverted by a network of enzyme-catalyzed reactions. While examples of these enzymes have been characterized in several different organisms, in plants most of those interconverting enzymes have yet to be identified. Using *Arabidopsis thaliana* as our model organism, our lab has identified, cloned, and characterized five of these flavin interconverting enzymes. AtFMN/FHy is a bifunctional enzyme, catalyzing both the ATP dependent phosphorylation of riboflavin to FMN (riboflavin kinase) and the hydrolysis of inorganic phosphate from FMN to form riboflavin (FMN hydrolase). It is presumed to be expressed in the cytosol. AtcpFHy1, a plastidial member of the haloacid dehalogenase superfamily, was found to also possess FMN hydrolase activity. We found two separate but closely related enzymes, AtRibF1 and AtRibF1, which both act as FAD synthetases, catalyzing the ATP dependent adenylation of FMN to form FAD. An additional FAD synthetase, AtFAD/XD, has been characterized and is localized to the cytosol. We have also been pursuing identification of the last unknown enzyme in the *de novo* riboflavin biosynthetic pathway, a phosphatase which can dephosphorylate 5-amino-6-ribitylamino-2,4(1*H*,3*H*) pyrimidinedione 5’-phosphate, thereby forming the substrate for lumazine synthase. To date, we have identified three candidate phosphatases capable of catalyzing this reaction. Identification of these enzymes has helped build a more complete picture of flavin cofactor metabolism.

POS-WED-288

MULTIPLEX MICRO-RESPIRATORY MEASUREMENTS OF ARABIDOPSIS TISSUES

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Researchers often want to study the respiratory properties of individual plants, parts of plants, or a population of mutants, in response to a range of treatments. Arabidopsis is an obvious model for much of this work, however, due to its size it represents a challenge for gas exchange measurements of respiration. Combining micro-respiratory technologies with multiplex assays has the potential to bridge this gap and make measurements in this model plant species. We show the adaptation of commercial technology used for mammalian cell respiration analysis to study three critical tissues of interest; leaf sections, root tips and seeds. Measurements of respiration in single leaf discs of 2.5 mm in diameter have allowed the age dependence of respiration rate in Arabidopsis leaves across the rosette to be observed. Oxygen consumption of single root tips from plate-grown seedlings show the enhanced respiration of root tips and their time-dependent susceptibility to salinity. Monitoring of oxygen consumption of single Arabidopsis seeds shows the kinetics of respiration over the first 48h post-imbibition and the effect of phytohormones GA and ABA, on respiration during seed germination. These studies highlight the potential for multiplexed micro-respiratory assays to study oxygen consumption in Arabidopsis mutant populations, in phenotypic screens and in ecotype comparisons.

POS-TUE-289

GENOME-SCALE CONSTRAINT-BASED *IN SILICO* MODELLING OF *ARABIDOPSIS THALIANA* ENERGY METABOLISM

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Genome-scale models of metabolic networks have been successfully used to describe the whole-cell metabolism of different organisms. This approach has immense potential to speed up the rate of discovery while reducing the need for expensive lab work and clinical trials, by allowing the exploration of phenotypic effects of gene knockouts, gene insertion, or up-regulation of gene expression directly *in silico*. Metabolic networks describe the entire set of biochemical reactions, physical processes and physiological states that regulate cellular life. They are sophisticatedly controlled biochemical reaction systems that can be dynamic, compartmentalized and responsive to perturbation. In this project, we combined omics data from disparate resources to build a comprehensive genome-scale model of Arabidopsis thaliana metabolism, with a focus on energy metabolism and intracellular compartmentation. First, we used localization information from the subcellular location database for Arabidopsis proteins (SUBA3, http://suba.plantenergy.uwa.edu.au) to develop accurate models describing *Arabidopsis* metabolism at an organelle level. After an extensive curation effort and quality control processes, these computational models were combined to form a global model, representing the metabolism of a generic cell. During the reconstruction process, we placed a particular emphasis on referencing all the models components to persistent databases. This systematic annotation allows the unambiguous identification of chemical compounds, reactions or genes and facilitates the maintenance and future expansions of the models. Reconstructions of individual compartments and the global cell models are readily available in the System Biology Markup Language. The model is capable of predicting fluxes under chosen constraints that correlate with experimental data and observations. The network reconstruction can form a solid basis for the development of a consensus model for *Arabidopsis thaliana* and become a valuable metabolic engineering tool to investigate plant metabolism.

POS-WED-290

A FUNCTIONAL STUDY OF *ARABIDOPSIS THALIANA* SIRTUINS

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Abiotic stresses are the major causes of yield loss in crops. These losses are the result of multiple mild stresses episodes throughout the growing season as well as periodical severe stresses. Therefore, breeding of crop varieties with improved responses to environmental changes is one of the important goals in modern agriculture. Crop varieties with enhanced tolerance to abiotic stress will broaden the window of optimal growth conditions for cultivated crops, thereby increasing average yield, yield stability and productive acreage. Such traits will provide substantial benefits to farmers and processors; may reduce costs in seed production and may lead to implementation of new strategies in plant breeding. Today the molecular and biochemical mechanisms involved in abiotic stress responses are still poorly understood and the signaling networks remain elusive. Recent data indicate that an efficient energy homeostasis contributes to stress tolerance and that the modulation of cellular NAD(P) homeostasis is an attractive and innovative strategy to improve plant performance in stress conditions. Functional studies in various eukaryotes indicate a potential important role for sirtuins in NAD+ dependent stress tolerance (Lagouge et al., 2006). Sirtuins catalyze protein and histone deacetylation and have been suggested to play a role in the suppression of recombination, chromosome stability, metabolic regulation and ageing (Denu et al., 2003). Through stress related phenotyping of transgenic plants with perturbed sirtuin levels, molecular phenotyping and protein-protein interaction studies, we provide new insights in the molecular function of sirtuins within the plant stress response.

POS-TUE-291

CHARACTERIZATION OF METHIONINE CYCLE ENZYMES MTI1 AND DEP1 IN *ARABIDOPSIS THALIANA*

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The Methionine (Met) or Methylthioadenosine (MTA) Cycle is a recycling pathway that converts MTA back to methionine. MTA is produced as a byproduct during ethylene, polyamine and nicotianamine synthesis. We previously identified 5-METHYLTHIORIBOSE-1-PHOSPHATE ISOMERASE1 (MTI1) and DEHYDRATASE-ENOLASE-PHOSPHATASE-COMPLEX1 (DEP1) as enzymes of the Met Cycle via yeast complementation. Analyses utilizing T-DNA Insertion mutants confirmed MTI1 and DEP1 as enzymes of this pathway in *planta*. Since MTI1 and DEP1 are involved in the recycling of Met, we tested the growth of *mti1* and *dep1* mutants under sulfur deficiency conditions. When grown on half-strength MS plates supplemented with 50µM or 100µM sulfate for extended periods of time the mutants showed stress symptoms and reduced growth. However, when grown at 500µM sulfate, normal MS medium or soil no phenotypic differences were observed. Additionally, hydroponic growth analyses with low sulfate concentrations were performed. Besides reduced root growth mutants showed severe reproductive defects such as short inflorescences, deformed flowers and missing siliques and seeds. Previous expression studies of Met cycle genes revealed their predominant expression in the vasculature of shoots and roots. Therefore, we analyzed the tissue specificity of MTA producing enzymes. In fact, the genes encoding for polyamine and nicotianamine synthases showed expression predominantly in vascular tissues. Since these enzymes consume Met as well as produce MTA the sulfur deficiency phenotype of *mti1* and *dep1* may be caused by breakdown of the Met/SAM pool and/or through inhibitory effects of accumulating Met cycle metabolites. Targeted metabolic analyses should provide further insight in this matter.

POS-WED-292

REGULATION OF SECONDARY SULFUR ASSIMILATION DURING DROUGHT STRESS IN *ARABIDOPSIS THALIANA*

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Secondary sulfur assimilation in Arabidopsis is responsible for the biosyntheses of secondary metabolites with important functions including hormones, such as brassinosteroids, and defense compounds such as glucosinolates (Chan et al 2013). Furthermore, I hypothesized that metabolites involved in drought stress may also act as retrograde signals from the chloroplast to the nucleus (Chan et al 2010). Indeed, we showed that a by-product of secondary sulfur assimilation, 3'-phosphoadenosine 5'-phosphate (PAP), which is degraded in the chloroplast, acts as a stress retrograde signal to the nucleus (Estavillo et al 2011). PAP accumulates in Arabidopsis during abiotic stresses such as drought and high light, is capable of moving from the chloroplast to the nucleus, and alters RNA metabolism to activate stress responses. Three research questions on secondary sulfur metabolism will be covered in this presentation: regulation of key secondary sulfur metabolism enzymes during drought, subcellular cross-talk between enzymes located in different compartments, and transport of PAP within the cell. Key secondary sulfur assimilation enzymes were found to be redox-sensitive; therefore redox control appears to be one of the mechanisms regulating PAP accumulation during drought. Transcripts of secondary sulfur assimilation enzymes located in different subcellular compartments are also differentially regulated during drought, suggesting compartment-specific coordination of secondary sulfur assimilation in drought. Finally, the roles of PAP transporters in abiotic stress are also examined.

POS-TUE-293

PSBS PROTEIN INTERACTIONS DURING NON-PHOTOCHEMICAL QUENCHING

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The non-photochemical quenching of excitation energy (NPQ) describes a photoprotective mechanism in the antenna of PSII which dissipates excess excitation energy as heat at the level of ^{1}Chl* and by that prevents the formation of singlet oxygen in PSII. Four different components contribute to NPQ, namely qT, qE, qZ and qI. qT represents the mechanism of 'state transitions' and seems to contribute to NPQ in higher plants only under low, non-saturating light conditions. Under saturating light conditions, the qE mechanism represents the dominant NPQ component in the short-term (up to 60 min). The qE component of NPQ is based on a complex mechanism, which is strictly dependent on the ΔpH across the thylakoid membrane, the PsbS protein and the xanthophyll zeaxanthin (Zx). According to the current understanding of qE, a low pH in the thylakoid lumen induces (i) PsbS-dependent conformational changes in the antenna of PSII and (ii) the formation of Zx, resulting finally in the quenching of excitation energy in PSII antennae. The central role of PsbS in these processes is related to the function of PsbS as sensor of the lumen pH. However, the molecular basis of this central function and particularly the underlying interactions of PsbS with PSII antenna proteins that lead to energy quenching are largely unclear. In this work, we present an in vivo approach using chemical crosslinking to identify protein interactions involving the PsbS protein during NPQ induction and relaxation in *Arabidopsis thaliana*, revealing its direct role in the formation of quenching sites in the antenna complexes of photosystem II.

POS-WED-294

DIFFERENTIAL REGULATION OF PLASTID PROTEINS IN STAY-GREEN ARABIDOPSIS: BEYOND THE RETENTION OF LHCII AND CHLOROPHYLL

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An individually darkened leaf model was used to study protein changes in the *Arabidopsis* mutant *stay-green* (*sgr1*) to partially mimic the process of leaf-covering senescence that occurs naturally in the shaded rosettes of *Arabidopsis* plants. Utilizing this controlled and predictable induced senescence model has allowed the direct comparison of *sgr1* with Col-0 during the developmental period preceding the retention of chlorophyll and light harvesting complex II (LHCII) in *sgr1* and the induction of senescence in Col-0. Quantitative proteomic analysis of soluble leaf proteins before the initiation of senescence has revealed a range of differences in plastid soluble protein abundance in *sgr1* when compared to Col-0. Changes were also observed in the membrane located machinery for photosystem II (PSII), in Calvin cycle components, proteins involved in redox control of the stromal compartment and ammonia assimilation that differentiated *sgr1* during the early stages of the senescence process. The changes in PSII abundance were accompanied with a lower capacity of photosynthetic CO2 assimilation in *sgr1* than Col-0 after return of plants to lighted conditions following 3 and 5 days of darkness. Together these data provide insights into the significant differences in the steady-state proteome in *sgr1* and its response to senescence, showing this cosmetic stay-green mutant is in fact significantly different to wild-type plants both before and during leaf senescence.

POS-TUE-295

DYNAMICS AND MECHANISMS OF ENERGY DISSIPATION

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The dissipation of excess light energy as heat (NPQ) represents an efficient photoprotective mechanism in plants. At least two different quenching sites are active in Arabidopsis under *in vivo* conditions: one is activated by the pH- and PsbS-dependent detachment and aggregation of a fraction of photosystem II (PSII) antenna proteins, while the other is based on the zeaxanthin (Zx)-dependent formation of a quenching site located in antenna proteins attached to the PSII reaction center. We have analyzed the dynamics of NPQ and Zx conversion upon illumination at different light intensities in plants grown under different growth light intensities. Our analyses provide evidence that more slowly (> 30 min) developing and relaxing NPQ processes are kinetically closely related to the dynamics of Zx formation and epoxidation in wild-type and PsbS-deficient plants. However, comparative analysis of the *pgr1* mutant (which is unable to acidify the thylakoid lumen below a pH of 6.0) and the xanthophyll cycle mutants *npq1* (deficient in Zx) and *npq2* (constitutively accumulating high levels of Zx) indicated, that similar slowly relaxing dissipative states are inducible also in absence of a low lumen pH and a functional xanthophyll cycle. Analyses of ultrafast chlorophyll fluorescence kinetics applied to high light acclimated plants indicated the activation of a novel, very efficient NPQ mechanism which is based on the interaction of PSII and PSI.

POS-WED-296

A COMPREHENSIVE GLOBAL TRANSCRIPTIONAL ANALYSIS OF CHLOROPHYLL CATABOLIC GENES

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The efficient light-harvesting properties of chlorophylls make them potential phototoxins since they can transfer absorbed light energy to oxygen resulting in the generation of reactive oxygen species. As a consequence, chlorophyll synthesis and degradation is tightly regulated throughout plant life. Chlorophyll degradation is known to occur during leaf senescence and in response to adverse environmental conditions that perturb the equilibrium of photosynthesis. In land plants, the highly conserved multistep pheophorbide a oxygenase (PAO) pathway has been identified as a major route for chlorophyll breakdown and most of the genes that function in the pathway have been identified. In order to further characterize the mechanism of chlorophyll degradation in plants a comprehensive transcriptional analysis was performed in Arabidopsis. A genome wide expression correlation analysis revealed that transcription of *PAO* is highly correlated with a specific subset of annotated chlorophyll catabolic genes (CCG) as well as a number of novel genes whose function can be plausibly related to chlorophyll degradation. A condition specific expression analysis, that included high-resolution temporal transcript profiling, revealed that expression of many of the coexpressed and annotated CCGs were found to be coordinately induced during senescence and in response to a range of abiotic and biotic stresses. A promoter content analysis of the coexpressed genes revealed an enrichment in a number of putative transcription factor binding sites. The high level of coexpression observed for the CCGs supports that transcriptional regulation plays a major role in coordinating chlorophyll degradation in Arabidopsis. Further, the highly similar transcriptional profiles support that a common chlorophyll degradation pathway is activated during senescence and in response to environmental stresses.

POS-TUE-297

ENGINEERING DROUGHT TOLERANCE IN BRASSICACEAE BY MANIPULATING THE SAL1 GENE

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SAL1 is a phosphatase that degrades 3'-phosphoadenosine-5'-phosphate (PAP), a by-product of sulfur metabolism. PAP accumulates in the wild-type *Arabidopsis* during drought. Accumulation of PAP can result in inhibition of exoribonucleases, which changes gene expression to activate stress responses in the plants. *Arabidopsis* lacking a functional SAL1 gene are more drought tolerant but, the *sal1* mutant has altered rosette morphology and delayed development when compared to the wild-type plants, which are agriculturally undesirable traits. The ultimate goal of my research is to investigate the possibility of improving drought tolerance in plants without affecting plant development by manipulating the SAL1 gene. Therefore, I intend to transiently reduce the SAL1 protein at near flowering stage and investigate whether the transient PAP increase will render drought tolerance without a growth penalty. I produced different constructs containing either *SAL1* cDNA, *SAL1*-targeting hair-pin RNAi or artificial miRNA, driven by various promoters - strong constitutive, embryo-to-germination stage specific, stress-responsive or dexamethasone-inducible. So far, work on *SAL1* hp constructs suggested that silencing a gene involved in regulating RNA metabolism using the hpRNAi system is not effective, due to feedback silencing of the hp insert. Consequently, current work is now focusing on 35S:amiRNA constructs as well as complementing *sal1* mutant with functional SAL1 at the embryo-to-germination stage. Additionally, I am investigating the role of SAL1, particularly during drought, in *Camelina sativa* and other Brassica species. Preliminary work indicates that there are two isoforms of SAL1 gene in *Camelina* and that, as in *Arabidopsis*, PAP levels also increase during drought.

POS-WED-298

A SURVEY OF DOMINANT MUTATIONS IN *ARABIDOPSIS THALIANA*

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Following the recent publication of a comprehensive dataset of 2,400 genes with a loss-of-function mutant phenotype in *Arabidopsis thaliana* (Lloyd and Meinke, Plant Physiol. 158: 1115-1129), questions remained concerning the diversity of dominant mutations in *Arabidopsis*. Most of these dominant phenotypes are expected to result from inappropriate gene expression, novel protein function, or disrupted protein complexes. This poster, which summarizes a recent review article published in Trends in Plant Science (Meinke, 2013), highlights the major classes of dominant mutations observed in model organisms and presents a collection of 200 *Arabidopsis* genes associated with a dominant or semidominant phenotype. Emphasis is placed on mutants identified through forward genetic screens of mutagenized or activation-tagged populations. These datasets illustrate the variety of genetic changes and protein functions that underlie dominance in *Arabidopsis* and may ultimately contribute to phenotypic variation in flowering plants.

POS-TUE-299

CHLOROPLAST FUNCTION DATABASE II: A LARGE-SCALE COLLECTION OF HOMOZYGOUS MUTANTS AND THEIR PHENOTYPE EFFECTS FOR NUCLEAR-ENCODED CHLOROPLAST PROTEINS

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The relationship between genotypes and phenotypes is important for systematic approach of functional genomics research. We have constructed the Chloroplast Function Database that has so far offered phenotype information on 1722 transposon- or T-DNA-tagged lines of the nuclear-encoded chloroplast proteins in *Arabidopsis*. Here, we present the development of the second version of the database, which was redesigned to increase the number of mutant characters and new user-friendly tools for data mining and integration. The upgraded database offers information on mutant screens for any visible phenotype against 2495 tagged lines to create a comprehensive homozygous mutant collection. The collection consists of 147 lines with seedling phenotypes and 185 lines for which we could not obtain homozygotes, as well as 1740 homozygotes with wild-type phenotypes. Besides providing search service about homozygous lines and lines that we could not confirm the T-DNA insertion at the flanking sequence positions, the database includes access to a link between gene locus and existing publicly available databases. In addition, high-resolution images of plastid morphologies of mutants with seedling-specific chloroplast defects as observed with transmission electron microscopy (TEM), are available in the current database. This trial was partially successful and we classified into eleven classes from plastid morphology which has a common functional trend to the feature of inner membrane structure. We also found a new unusual structure of plastid which cannot be seen from the morphology of whole plant, suggesting that our TEM observation is a useful approach for screening of novel mutant. Thus, our upgraded database is a useful and comprehensive information resource that can help researchers to connect individual Arabidopsis genes to plastid functions on the basis of phenotype analysis of our tagged mutant collection. It can be freely accessed at http://rarge.psc.riken.jp/chloroplast/.

POS-WED-300

ORGANELLAR PROTEIN TRAFFICKING AND INSERTION OF TAIL-ANCHORED PROTEINS IN PLANTS

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An estimated 20-30% of all open reading frames encode integral membrane proteins. Despite this large number, the process by which they are targeted and inserted into membranes is incompletely understood. A subset of integral membrane proteins are tail-anchored (TA), meaning their targeting information resides on the C-terminus of the protein which contains a single short C-terminal transmembrane domain (TMD). In yeast, TA proteins in the secretory pathway are recognised by a cytosolic Guided Entry of Tail anchored proteins 3 (GET3) protein, which docks with the GET1/GET2 complex for protein insertion into the endoplasmic reticulum (ER) membrane in a GET4/5-dependent manner. In plants, the acquisition of plastids has meant that a higher degree of specificity is required for TA protein targeting. In Arabidopsis, three putative orthologs of GET3 (At1g01910; AtGET3-1, At3g10350; AtGET3-2 and At5g60730; AtGET3-3) are encoded in the genome. AtGET3-1 is cytosolic and shares higher sequence similarity to the yeast GET3 than the other two orthologs. AtGET3-2 and AtGET3-3 localise to the stroma of plastids and the outer mitochondrial membrane respectively. Using a combination of In vitro protein insertion and fluorescent tagging we have analysed the function of the mitochondrial isoform leading us to propose a new model for TA protein targeting in Arabidopsis thaliana.

POS-TUE-301

CAM-REGULATION OF CNGCS IN *ARABIDOPSIS*: NEW INSIGHTS AND NEW MODELS

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Cyclic nucleotide-gated channels (CNGCs) are nonselective cation channels involved in biotic and abiotic stress responses, development and ion homeostasis. Here, we analysed the molecular mechanism of calmodulin(CaM)-binding to CNGC20, a channel expressed in e.g. guardcells and epidermal cells. We showed that CNGC20 from *Arabidopsis thaliana* binds CaM in a Ca^{2+}-dependent manner and interacts with all AtCaM isoforms but not with the CaM-like proteins CML8 and CML9. CaM interaction with the full-length channel was demonstrated in planta, using bimolecular fluorescence complementation. This interaction occurred at the plasma membrane, in accordance with our localisation data of GFP-fused CNGC20 proteins. The CaM binding site was mapped to an isoleucine glutamine (IQ) motif, which has not been characterised in plant CNG channels so far, but it is not required for plasma membrane targeting of the full-length channel. Our results show that compared to the overlapping binding sites for cyclic nucleotides and CaM in CNG channels studied so far, they are sequentially organised in CNGC20. We will also present latest results on CaM-binding properties of other CNGCs with different CaM selectivities/affinities. We will discuss new insights on CNGC regulation by CaM underlining the functional diversity within this gene family.

POS-WED-302

BIOCHEMICAL CHARACTERIZATION OF OSERG3 AS A SMALL C2-DOMAIN PROTEIN IN RICE

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The C2 domain is known as a Ca^{2+}-dependent membrane-binding motif found in many cellular proteins involved in signal transduction or membrane trafficking in eukaryotes. OsERG3 has been identified as a homolog of OsERG1, which is a small C2-domain protein consisting of a single C2 domain. To characterize the biochemical properties of OsERG3, its Ca^{2+}- and phospholipid-binding activities have been analyzed in this study. Based on our results, it has been proved that OsERG3 is unable to interact with phospholipids in either the presence or absence of Ca^{2+} ions. Nonetheless, the OsERG3 protein showed calcium-binding activity in an in vitro $^{45}Ca^{2+}$-binding assay which is not observed with OsERG1. However according to the results of native polyacrylamide gel electrophoresis (PAGE) and glutaraldehyde cross-linking experiments, both OsERG1 and the OsERG3 proteins showed oligomerization properties in solution. Taken together it has been suggested that OsERG3 is a cytosolic small C2-domain protein which sustains Ca^{2+}-binding and oligomerizing properities in rice cells.

POS-TUE-303

POST-TRANSCRIPTIONAL REGULATION BY INITIATION CONTEXT IN *ARABIDOPSIS THALIANA*

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Nucleotide sequences around translational initiation site (TIS) are important cis-acting element for post-transcriptional regulation. Although the Kozak sequence (RccAUGG) is known as the necessary element for translation, sequence context around TIS varies widely diversity among different organisms. Through the 21 nucleotides 5'-UTR triple nucleotide substitution assay, we revealed positional effect of each nucleotide around TIS in Arabidopsis Thaliana. Adenine residues in -5 to -1 region were necessary for translation, but they were not favorable when they break intrinsic sequence in remaining region. GT rich 5'-UTR could inhibit the translation but it could be complemented when 5 to -1 region was substituted with triple Adenine. These observations support that each intrinsic sequence context in front of TIS has own role in regulation of translation and serial adenine was only favorable in -5 to -1 region in the transcript of Arabidopsis Thaliana.

POS-WED-304

ANALYSIS OF THE *ARABIDOPSIS THALIANA* NUCLEAR PROTEOME

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High throughput technologies have opened unprecedented possibilities of studying molecular processes at the global level. However, so far such strategies have not been used for global analysis of Arabidopsis nuclear proteome, most likely because of serious problems with efficient isolation of intact Arabidopsis nuclei. We have applied shotgun proteomics to create a currently most detailed catalog of nuclear proteins of Arabidopsis T-87 suspension cells. Nuclei were isolated in six replicates by a gradient centrifugation followed by in-solution digestion or Filter Aided Sample Preparation (FASP). Subsequently, samples were analyzed with nanoLC coupled with the Orbitrap Velos mass spectrometer. The use of high-high method allowed the acquisition of both MS and MS/MS spectra with a high resolution provided by Orbitrap. To obtain high quality results, we not only performed a peptide-protein identifications on Mascot server supported by the TAIR10 database, but also estimated a false discovery rate with Percolator and MScan software. The final data were organized into a database equipped with web interface allowing users to change identification parameters and downloading lists of proteins. Each protein was identified based on at least two different peptides and the peptides of identified proteins had to be found in at least three biological replicates. We have successfully identified 6753 different proteins, including products of alternative gene splicing, albeit the distinguishing between different splicing variants of many proteins was not possible. Upon grouping of alternative splice variants, we have eventually identified the protein products of 4862 genes.

POS-TUE-305

STABLE EXPRESSION OF THE SWEET PROTEIN MONELLIN VARIANT MNEI IN TOBACCO CHLOROPLASTS

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Monellin is a naturally sweet protein that consists of two polypeptide chains and has potential uses as a highly potent non-carbohydrate sweetener. We aimed to make this protein more usable by increasing its stability and expressing it in a high-yielding system. MNEI is a modified version of the protein that consists of the two natural chains of monellin joined via a dipeptide linkage. In the thermostability analysis of MNEI variants, four mutated MNEIs, MNEI-E24L, MNEI-E24F, MNEI-E24W, and MNEI-E24A, had higher melting temperatures than wildtype MNEI and retained their sweet flavor even at temperatures above 70 °C. Our findings indicate that the increased stability of monellin allows it to retain its strong sweetness even under extreme conditions. We successfully overexpressed the thermostable MNEI mutants in tobacco chloroplasts. Here, we report that the MNEI mutants showed enhanced thermostability, and the stable forms of MNEI can be produced through plastid transformation in tobacco.

POS-WED-306

PROTEOME COVERAGE OF ARABIDOPSIS: IMPLICATIONS FOR SHOTGUN PROTEOMIC STUDIES

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The recent aggregation of matched proteomics data for the model plant Arabidopsis has enabled the assessment of a diverse array of large scale shotgun proteomics data. A collection of over nine million matched peptides was used to assess proteome coverage and experimental parameters when compared to the theoretical tryptic peptide population. The analysis indicated that the experimentally identified median peptide mass was significantly higher than the theoretical median tryptic peptide in Arabidopsis. This finding led to a critical examination of precursor scan ranges currently being employed by shotgun proteomic studies. The analysis revealed diminishing returns at the high end scan range and opportunities for greater coverage and identifications at the low mass range. Based on these findings, a recommended basic scan range of 300 to 1200 m/z would suitably capture the peptide population in shotgun proteomic analyses in Arabidopsis.

POS-TUE-307

CHARACTERISATION OF GLYCINE MAX SYMBIOSOME MEMBRANE IRON TRANSPORTERS

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The legume-rhizobia symbiosis are noted for their ability to utilize atmospheric N and their contribution to sustainable agricultural systems by reducing the use of nitrogen fertilizer. The symbiotic N fixation takes place in nodules, where the rhizobia are enclosed by a membrane of plant origin, termed the symbiosome membrane (SM). Within the specialized low oxygen environment, the differentiated bacteria (bacteroids) supply the host with N in a biologically available form and are totally dependent on their hosts for many nutrients, including reduced carbon as a major energy source and many other essential trace elements. The nutrient exchange through the interface between rhizobia and host nodule cells is essential for nitrogen fixation and plant growth under low nitrogen conditions but is poorly understood. Micronutrients such as iron are essential as part of key proteins involved in N_2 fixation such as nitrogenase and cytochromes used in the bacteroid electron-transport chain. However iron is usually in its oxidized insoluble form and therefore is not easily acquired by the plant. My study focused on the characterization of four members of the *Glycine max* NRAMP/DMT metal transport family by yeast complementation and analysis of their expression in response to iron deficiency/sufficiency. All four genes were highly expressed in nodule with two showing increased expression when plants were grown in iron deficient conditions and two showing increased expression when grown in high iron conditions. Yeast complementation suggests all four genes can transport iron although one showed stronger complementation of the iron transport mutant.

POS-WED-308

ATSERPIN1, AN INHIBITOR IN VIVO OF THE PAPAIN-LIKE CYSTEINE PROTEASE RD21

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Proteins of the serpin family are ubiquitous in the Plant Kingdom. Serpins feature an exposed reactive center loop (RCL), which displays an amino acid sequence that serves as a protease bait. RCL cleavage of inhibitory serpins by a target protease results in irreversible formation of a covalent serpin-protease complex. The Arabidopsis genome contains eight genes that encode full-length serpins. Our recently solved structure of the stressed conformation of AtSerpin1 from Arabidopsis, which has the plant species-conserved reactive center P2–P1' Leu-Arg-X (where X = small residue), displays both conserved and plant-specific serpin features. The electrostatic surface potential below the RCL was found to be highly positive, whereas the breach region critical for RCL insertion was an unusually open structure. AtSerpin1 accumulated in plants as a full-length and a cleaved form. Fractionation of seedling extracts by non-reducing SDS-PAGE revealed the presence of an additional slower migrating complex that was absent when leaves were treated with the specific cysteine protease inhibitor E-64. Significantly, RESPONSIVE TO DESICCATION-21 (RD21), a papain-like cysteine protease, was the major protease labelled with the E-64 derivative DCG-04 in wild-type extracts but not in extracts of mutant plants constitutively overexpressing AtSerpin1, indicating competition. Fractionation by non-reducing SDS-PAGE followed by immunoblotting with RD21-specific antibody revealed that the protease accumulated both as a free enzyme and in a complex with AtSerpin1. Importantly, both RD21 and AtSerpin1 knock-out mutants lacked the serpin-protease complex. The match between AtSerpin1 and RD21 is reminiscent of the inhibition of cathepsins K, L and S by the Clade-B mammalian serpin, SCCA-1 (SERPINB3).

POS-TUE-309

ARABIDOPSIS BLUE LIGHT RECEPTOR, PHOTOTROPIN 2, IS A SUBSTRATE FOR MODIFICATION BY SUMO

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SUMOs (Small Ubiquitin-related MOdifiers) are small proteins that reversibly modifiy other proteins, changing their interactions, activities, localization and stability. In plants, sumoylation has been implied in many processes e.g. flowering or hormone signaling, but no links with light perception have been reported. We investigated if phototropin2, the plant blue light receptor, can be a target of sumoylation and if it can interact with SUMOs and their ligases. In silico analysis of the phototropin2 sequence revealed numerous potential sites of modification by SUMO. The N-terminal part of phot2 was sumoylated when expressed in *E. coli* together with SUMO and its activating and conjugating enzymes. The modification was observed without E3 ligase and enhanced in the presence of MMS21 SUMO ligase. The phot2 sumoylation pattern was isoform-specific: SUM1 and SUM2 formed poly- or multi-sumoylated products. In the case of SUM3 most of the protein was monosumoylated and no modification was detected with SUM5. MS/MS analysis of SUM3 adduct revealed several sumoylation sites localized in different areas of the protein. Interactions between phot2 and the sumoylation machinery were studied in planta by transient expression in *Nicotiana benthamiana* epidermal cells. Bimolecular fluorescence complementation analysis showed interactions between phot2 and SUM1, SUM3 and MMS21. The interactions were further tested using the yeast two hybrid system. In order to investigate the physiological function of phototropin2 sumoylation, several *Arabidopsis* knock-out mutants in genes related to SUMO conjugation were analyzed for their chloroplast relocation, the response mediated by phototropin2. Whereas most mutants tested showed wild-type reaction parameters, siz1 plants exhibited increased sensitivity to short light pulses. To sum up, phototropin2 is a substrate for sumoylation and this process may affect signaling from this photoreceptor.

POS-WED-310

PSKR1 IS A MOONLIGHTING RECEPTOR GUANYLATE CYCLASE THAT ACTS AS A HOMO DIMER

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Cyclic GMP is a second messenger that is generated through guanylate cyclases (GCs) by the conversion of GTP to cGMP. Using search motifs based on functionally assigned amino acids in catalytic domains of known GCs we identified candidate plant GCs including a novel class of GC-linked receptor kinases one of which is the phytosulfokine receptor 1 (PSKR1). PSKR1 is a membrane-localised leucine-rich repeat receptor-like kinase that also possesses intrinsic guanylate cyclase (GC) activity which is conferred by a GC catalytic centre that is embedded within its kinase domain. The recombinant cytoplasmic domain of PSKR1 has both guanylate cyclase and kinase activity in vitro (Kwezi et al. 2011 J Biol Chem 286: 22580-8). Structural analysis (Misono et al. 2011 FEBS J 278: 1818-720) predicts that dimerization of PSKR1 is necessary to form a complete catalytic site essential for GC activity. Using size exclusion chromatography and analytical ultra centrifugation sedimentation studies we show that PSKR1 can form transient homo-dimers in vitro. Subsequent cloning of the cytoplasmic domain sequence into BiFC (split eYFP) vectors (Chakrabarty et al. 2007 MPMI 20: 740-50) expressed in bacteria (BL21-AI) showed that PSKR1 forms homo-dimers interacting at the C terminal end in bacteria. Based on homology with other plant receptor like kinases that are also GC-linked receptor kinase such as the brassinosteroid receptor BRI1 (Kwezi et al. PLoS one 2: e449), we predict that PSKR1 naturally forms hetero-dimer complexes and this is currently being tested in planta.

POS-TUE-311

RAPID AND FACILE EMS MUTANT IDENTIFICATION BY A SINGLE PARENTAL BACKCROSS AND WHOLE GENOME SEQUENCING

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Forward genetic screens remain a cornerstone for defining gene function. However, to locate a causal mutation, the practice of crossing to a polymorphic background to generate a mapping population can be problematic if the phenotype is masked in the hybrid F2 progeny, or dependent on additional parental specific traits. Here in a screen for leaf hyponasty mutants, we have performed a single backcross of an Ethane Methyl Sulphonate (EMS) generated hyponastic mutant to its parent. Analysis of a bulked homozygous F2 population by use of Next-Gen-Sequencing (NGS) unambiguously determined the causal mutation to reside in *HASTY*, a previously characterised gene that results in hyponasty when mutated. In this instance, we did not need to resort to additional approaches, such as targeted deep re-sequencing or genetic methods to discriminate the casual mutation. The simplicity of performing a single parental backcross and genome sequencing a small pool of segregating mutants has great promise for identifying mutations that may be difficult to map using conventional approaches.

POS-WED-312

INID: AN AUTOMATIC FRAMEWORK FOR IDENTIFYING NETWORK MODELS FOR INTERPLAYS AMONG DEVELOPMENTAL SIGNALING IN PLANTS

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Integration of internal and external cues into developmental programs is indispensable for growth and development of plants, which involves complex interplays among signaling pathways activated by the internal and external factors (IEFs). However, decoding these complex interplays is still challenging. Here, we present a web-based platform that identifies key regulators and Network models delineating Interplays among Developmental signaling (iNID) in plants. iNID provides a comprehensive resource of 1) transcriptomes previously collected under the conditions treated with a broad spectrum of IEFs and 2) protein and genetic interactome data. In addition, iNID provides an array of tools for identifying key regulators and network models related to interplays among IEFs using transcriptome and interactome data. To demonstrate the utility of iNID, we investigated the interplays of 1) phytohormones and light and 2) phytohormones and biotic stresses. The results revealed 34 potential regulators of the interplays, some of which have not been reported in association with the interplays, and also network models that delineate the involvement of the 34 regulators in the interplays, providing novel insights into the interplays collectively defined by phytohormones, light, and biotic stresses. Therefore, iNID serves as a useful tool to provide a basis for understanding interplays among IEFs. iNID is available at http://sbm.postech.ac.kr/inid.

POS-TUE-313

A NOVEL APPROACH FOR UNDERSTANDING METABOLIC SYSTEM: METABOLOMICS-BASED MATHEMATICAL MODELING

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Understanding metabolic reaction networks comprising of enzymatic reactions and their regulatory mechanism is becoming more and more important for synthetic biology and metabolic engineering. Recently, it has become possible to acquire a large metabolome dataset from high-throughput instruments. Time-series metabolome data includes important information to understand metabolism as a system. The present work proposes a new technique for in silico analysis of a metabolic reaction network by using time-series metabolome data. In this approach, a mathematical model is constructed in the framework of Biochemical Systems Theory based on available information on metabolic pathways, which are composed of chemical reactions and feedback regulations by metabolites. The parameters in the model, namely, kinetic orders and rate constants, are estimated from actual time-series data of metabolite concentrations. The obtained mathematical model enables us to simulate metabolic behaviors and conduct the system analysis of a metabolic reaction network. In this presentation the result of our on-going study will be introduced.

POS-WED-314

DECIPHERING AND PREDICTION OF TRANSCRIPTOME DYNAMICS UNDER FLUCTUATING FIELD CONDITIONS

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Recent advances in plant molecular biology have revealed large effects of the circadian clock, organism age, and environmental stimuli on transcriptomes under simple, controlled laboratory conditions. However, the factors that control transcriptomes under natural conditions are largely unknown. We have developed statistical models using extensive field transcriptome data and the corresponding meteorological data. Here we show that the transcriptome dynamics of rice leaves in a paddy field were mainly governed by ambient temperature and endogenous diurnal rhythms, as well as by plant age and solar radiation. We also found diurnal gates for environmental stimuli, detected associations between the thresholds for plant response to solar radiation and signal-to-noise ratios for day-length change, and predicted transcriptomes under given environmental conditions. Our models comprehensively describe transcriptome dynamics under complex field conditions and will help researchers to translate the vast molecular knowledge amassed in laboratories into solutions to problems in agricultural and natural environments. Nagano A.J. et al., (2012) Cell. 151 (6), 1358-1369.

POS-TUE-315

RICE DB: AN ORYZA INFORMATION PORTAL LINKING ANNOTATION, SUB-CELLULAR LOCATION, FUNCTION, EXPRESSION, REGULATION AND EVOLUTIONARY INFORMATION FOR RICE AND ARABIDOPSIS

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Omics research in rice (*Oryza sativa*) relies on the use of multiple databases to obtain different types of information to define gene function. We present Rice DB: From Genes(s) to Function(s), which is a functional genomics database, linking gene loci to comprehensive annotations, expression data, and sub-cellular location of encoded proteins. Rice DB has been designed to integrate direct comparison of rice to Arabidopsis (*Arabidopsis thaliana*), based on orthology or "expressology", thus utilising and combining available information from two pre-eminent plant models. To establish Rice DB, gene identifiers (>40 types) and annotations from a variety of sources were compiled, functional information based on large-scale and individual studies was manually collated, hundreds of microarrays were analysed to generate expression annotations, and the occurrences of potential functional regulatory motifs in promoter regions were calculated. A range of computational sub-cellular localisation predictions were also run for all putative proteins encoded in the rice genome, and experimentally-shown protein localisations have been collated, curated and linked to functional studies in rice. A single search box allows anything from gene identifiers (for rice and/or Arabidopsis), motifs sequences, sub-cellular location, to keyword searches to be entered, with the capability of Boolean searches (such as AND/OR). To demonstrate the utility of Rice DB, a rice mitochondrial proteome was determined and compared with Arabidopsis, revealing conservation in sub-cellular location, expression and regulation between rice and Arabidopsis.

POS-WED-316

REDESIGNING PPR PROTEINS TO BIND NEW RNA TARGETS

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PPR proteins belong to a large family of RNA-binding proteins found in all eukaryotes, but particularly prevalent in plants. They play key roles in plant development, crop breeding and can be associated with mitochondrial disorders in humans. PPR proteins are essential for the expression of genes required for the construction and function of the major protein complexes involved in photosynthesis and respiration. They are thus vital during germination and early seedling development and some are absolutely required for autotrophic growth. The recent discovery of a code describing how PPR proteins recognize their target RNAs (Barkan et al., 2012) is a major breakthrough as it allows us to predict binding sites and construct custom-designed proteins to bind desired targets. In theory, we ought to be able to design factors capable of specifically altering expression of target transcripts by binding to them. To test this, we are working with RPF proteins (RNA processing factors), as they are closely related to CMS restorer genes that are known to block expression of specific mitochondrial transcripts. The new PPR-RNA target combinations are tested experimentally in vitro by electrophoretic mobility shift assays, giving a simple semi-quantitative measure of binding affinity. The validated combinations will be tested in vivo, after expressing the new PPR proteins in plants, in order to test the effects of these changes on organelle gene expression. This system has potential for many biotechnological applications in plant breeding and medicine.

POS-TUE-317

GENOME EDITING IN HIGHER PLANTS; TOWARD PRECISE MANIPULATION OF NUCLEAR AND ORGANELLE GENOMES

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Mutations either naturally occurring or induced by mutagens have been widely used for understanding its gene function, and also utilized when its gene function is changed and it is useful. However, such mutations are randomly occurred, and the screening the trait and its fixation are quite laborious and time-consuming. To overcome these problems, a new technology for genetic engineering, so called genome editing has been establishing. A key concept of genome editing is the target gene-specific double-stranded DNA break (DSB) by using custom designed endonucleases. On the target-specific DSB by the custom designed endonuclease(s), gene targeting and/or site-directed mutagenesis can be occurred efficiently at the DSB site. Especially, introducing and expressing the endonuclease(s) are simply needed for non-homologous end joining (NHEJ)-mediated site-directed mutagenesis (SDM), and thus it should replace classical methods such as mutagenesis approaches randomly produced or gene silencing techniques. To show the proof-of-concept demonstration for NHEJ-mediated SDM in higher plants, the engineered zinc finger nucleases were expressed to digest on the *ABI4* gene transiently in *Arabidopsis* cells, and it was confirmed that mutations occurred at the target site specifically and *Arabidopsis* mutant lines could be produced efficiently. I currently expand the usage of up-coming genome editing tools such as transcription activator-like nucleases and CRISPR/CAS for SDM in higher plants. In this presentation, the current status for genome editing in higher plants will be summarized, and it will also be discussed about the role of genome editing for precise manipulation of both nuclear and organelle genomes in higher plants.

POS-WED-318

RECONSTRUCTION OF LOW-FOLD SEQUENCED RECOMBINANT GENOMES USING A HMM APPROACH REVEALED NEW FLOWERING TIME RELATED GENES IN *FRAGARIA VESCA*

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Whole-genome sequencing of hundreds of individuals is requires substantial sequencing investment unless the focal genomes are sequenced sparsely only. Here we introduce a novel pipeline for whole-genome reconstruction from sparse sequencing, based on a Hidden Markov Model (HMM) approach. This HMM implements states for each parental and heterozygous genotype, transition and emission probabilities are estimated by an individual fit of a beta mixture model of their sparse genotypes representation. With this approach each genome is reconstructed by sample-specific HMM model. This adjusts the model to sample-specific features like coverage and sequencing errors. Additional filtering for mis-aligned reads reduced the error rate of wrongly assessed genotypes. Those reads introduce wrong allele frequencies, which increase the probability for wrong genotype assignment. Simulations studies revealed an extremely low error rate of our method, which was increased towards the telomeres. Applied on low-fold sequencing data derived from a 40 individuals F2 mapping population between *F. vesca Hawaii-4 x SD F. vesca tlf1*, we were able to identify three new QTLs. The QTLs were associated to not more than 7 candidate genes, each of which showed an effect on TFL1.

POS-TUE-319

UNCOVERING TRANSCRIPTIONAL CIRCUITS BY FUNCTIONAL GENOMICS

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Extensive genome-wide transcript profiling suggests that transcriptional regulation of gene expression plays an important role in the control of plant physiology. In this context, transcription factors (TFs) should be critical components that control almost every biological process and ultimately overall plant fitness and performance. While genetics and genomics studies uncovered some regulators of specific genes, most TFs are still not associated to a specific function. Likely, discovery approaches are significantly limited by the redundant nature of transcriptional circuits and TF protein families. To circumvent this problem, we implemented a TF-centered yeast one-hybrid (Y1H) screen as an alternative mean to uncover direct regulators of a key Arabidopsis clock component (CCA1). The strategy was instrumental in identifying a novel clock component (CHE) that directly regulates CCA1. Subsequent screens that uncovered novel regulators of other important biological processes further validated the approach. Thus, to generate a versatile research tool for the community we aimed to expand the initial 200 TF-collection to all predicted Arabidopsis transcriptional regulators. Here, we present the construction of the most comprehensive fully sequence-validated array of Arabidopsis TFs. This 1956-clone collection includes ~80% of all TFs predicted by most TF-specific databases and provides a significant clone number increase compared to previous publicly available resources. Furthermore, the collection is completely homogeneous, as all clones were generated following the same cloning strategy, and compatible with recombination based cloning. Thus, TF-coding sequences can be uniformly transferred to other vectors for downstream applications. Following this strategy a parallel collection of TF-constructs suitable for Y1H screens was generated. Using this daughter collection and an improved procedure to perform high-throughput Y1H automated screens, we identified novel regulators of CCA1. In summary, the resource presented here provides a comprehensive toolset for the discovery of direct transcriptional regulators for any Arabidopsis gene.

POS-WED-320

PROMOTERCAD: DATA DRIVEN DESIGN OF PLANT REGULATORY DNA

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Synthetic promoters that can control when, where, and how much of any gene gets expressed in any organism need to be developed for a wide range of bio-engineering purposes. PromoterCAD is a web application for designing synthetic promoters with altered transcriptional regulation. PromoterCAD takes a data-first approach, using published high throughput expression and motif data to guide DNA design. Employing such datasets for Arabidopsis thaliana, we demonstrate data mining tools for finding motifs for extreme expression, circadian oscillations, and tissue specific expression patterns. PromoterCAD uses the LinkData open platform for data publication and rapid web application development. This allows new data to be easily added, and the source code modified to add new functionality. PromoterCAD URL: http://promotercad.org.

POS-TUE-321

SPATIAL OPERATION OF ANTHER SPECIFIC PROMOTER(GALCHS7) USING RIP (RIBOSOMAL INACTIVATING PROTEIN) GENE THROUGH MICROSCOPIC ANALYSIS IN PETUNIA

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Previously, we isolated gALCHS7 promoter (1584 bp) from lily(Lilium hybrid cv. 'Acapulco') and introduced to Petunia hybrida cv. DreamRed by Agrobacterium-mediated transformation. Fluorometric GUS assays of individual organs demonstrated that gALCHS7 promoter showed highest GUS activity in anther but some transgenic plants (five of ten lines) also showed weak GUS expression in ovary. GUS activity of gALCHS7 promoter was revealed higher than that of 35S CaMV promoter (pBI121) by 1.5 to 3 folds. From the microscopic observation, red-signal of gALCHS7 promoter was expressed in pollen, endothesium and epidermis in all transgenic lines. But several transgenic plants also produced signal in ovules. No distinctive expression signal was detected in other organ through all satge. To identify the spatial expression of the promoter, we fused cDsRIP2 cDNA (Genebank AF219237, 831bp) as a cytotoxin gene to gALCHS7 promoter. Out of twenty-one independent transformants, fourteen transgenic petunia plants were contained RIP gene with genome PCR analysis. Among them, anther and ovule of two transgenic lines (10, 11) were showed RIP expression in RNA level. Pollen of these lines were not germinated as expected. We need more experiment for uncover spatial operation in anther and alternation of ovule in transgenic lines using microscopic analysis.

POS-WED-322

DIRECT MASS SPECTROMETRY IMAGING OF INTACT TISSUES OF *ARABIDOPSIS THALIANA*

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Recently MALDI mass spectrometry imaging (MSI) has been a powerful tool to map spatial distribution of molecules on the surface of biological materials. Frequently MSI has been applied to animal tissue slices to map various biological molecules on the slice, however most recently, it has been also applied to thin slice of plant tissues. We have been developing Ultra-high-resolution and ultra-high-accuracy mass spectrometer dedicated for imaging mass spectrometry of plant tissues and reported the successful MSI results coming from thin slice of young leaf of Arabidopsis thaliana last year. Because it has been still very difficult to make thin slices from Arabidopsis thaliana tissues, we tried to glue small intact tissues of Arabidopsis thaliana, such as leafs, roots or sprouts, onto a small transparent ITO-coated slide glass instead of thin slice of the tissues. The intact tissues were then vacuum dried and matrix substance was applied by sublimation prior to mass spectrometry imaging experiment. The tightly focused UV-LASER beam was irradiated, inside the vacuum chamber of the mass spectrometer, to make ions from matrix-coated sample surface and m/z of the ions were measured by commercial FTICR-MS (Bruker Daltonics Inc.; Apex-Qe-94T). Molecular ions of various metabolites including glucosinolates and anthocyanins were observed and their spatial distribution in the tissues was mapped successfully. We also found that some of the metabolites ions were even observed when focused UV-LASER was irradiated onto the non-matrix coated surface of dried Arabidopsis tissues. From the LDI (Laser Desorption Ionization) - MSI experiments, we found the spatial distribution of several metabolites was changed in the tip of the root after auxin treatment.

POS-TUE-323

SHORT-TERM RESPONSE OF AMINO ACID METABOLISM TO PHOSPHATE STRESS

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Phosphate is essential for plant nutrition, but its tendency to absorb to soil particles makes it poorly bioavailable. Prolonged phosphate deprivation ultimately leads to the development of a larger root system with improved phosphate acquisition capacity, inhibits shoot growth, and affects numerous aspects of carbon and nitrogen metabolism. Such phenotype develops only after the vacuolar Pi reserves have been consumed, typically several days after growth in low Pi. However, recent studies in several plant species have shown that numerous enzymes involved in primary metabolism are transcriptionally controlled within minutes to a few hours after removal of phosphate. N assimilation is also inhibited and amino acids accumulate in leaves before tissue Pi levels are significantly affected. We used 15N metabolic labelling to follow changes in the amino acid metabolism of barley over the course of the first 6 hours of Pi stress. Within an hour after the removal of phosphate from the nutrient solution, we observed a significant but transient increase in the pool size of numerous amino acids, coinciding with transient changes in photosynthetic parameters that are usually observed days to weeks after P stress. Flux analysis revealed that P stress rapidly affected primary N assimilation, photorespiration and de novo synthesis of amino acids which derive their C-skeletons from pyruvate and TCA cycle intermediates. Such metabolic adaptations appear conserved across monocots and dicots exposed to long-term P-starvation and the present study points to the requirement of rapid metabolic control over amino acid metabolism in the development of an appropriate early P-stress response.

POS-WED-324

PROTEOMIC ANALYSES OF RESPIRATORY METABOLISM UNDER SALINITY STRESS

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This work explores one approach to translational research. The molecular functions of many proteins are causally linked to stress tolerance through transgenic experiments in Arabidopsis. This knowledge could inform crop improvement strategies, by positioning homologous genes in crop species as potential targets in breeding programs. However, the genetic complexity of hexaploid bread wheat complicates matching Arabidopsis genes to specific genetic loci in wheat. Soil salinity constrains crop yields in many agricultural regions. Despite extensive scientific study of NaCl-treated plants over several decades, the effects of salinity stress on cellular metabolism are not fully characterised. Respiration plays an important role in a range of physiological processes deployed to cope with salinity, and this work examines the protein-level changes exhibited by mitochondria isolated from salt-treated wheat plants. Key results relate to the NaCl-linked induction of alternative oxidase (AOX), manganese superoxide dismutase (MnSOD), aconitase (Aco), and glutamate dehydrogenase (GDH). These changes were assessed at the protein-level in shoot and root tissue using quantitative proteomics. Protein abundance profiles were compared across tolerant versus sensitive wheat varieties. Although mitochondrial proteomes exhibit high similarity between varieties, this work documents higher abundance of MnSOD, β-cyanoalanine synthase (β-CAS), and serine hydroxymethyltransferase (SHMT) in tolerant lines. Regarding translational research, several of these proteins are causally linked to salinity tolerance in the Arabidopsis literature. This is evidenced by salt-tolerant and salt-sensitive phenotypes of Arabidopsis seedlings where homologous genes had been transgenically manipulated, either through knockout or overexpression. This work aims to fill a gap in translational research strategies, by linking results from the Arabidopsis literature with particular genetic alleles associated with tolerance in hexaploid wheat.

POS-TUE-325

HOW SIMILAR ARE THE NITRATE UPTAKE SYSTEMS OF ARABIDOPSIS AND CEREAL CROPS?

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The primary nitrogen source for cereal crops is nitrate (NO_3^-), of which a large proportion acquired by plants from soil is actively transported via members of the NRT families of NO_3^- transporters. Most of what we have learned of the NO_3^- uptake system to-date has been acquired from work with Arabidopsis and primarily from characterisation of the system in response to short-term perturbations which elicit the 'primary NO_3^-response'. Our analyses of cereals indicate fundamental differences between Arabidopsis and the cereal species not only in the gene number and family structure of all three families of *NRT* transporters, but also in the transcript abundance of the cereal *NRT* genes in response to NO_3^- provision in the context of the 'primary NO_3^-response' and over the entire lifecycle. Transcript abundance of *NRT2* genes encoding high-affinity NO_3^- transporters were highly correlated with NO_3^- uptake capacity across growth stages in cereals, whereas *NRT1* genes encoding low-affinity NO_3^- transporters were not. This response has yet to be observed in longer term, steady-state Arabidopsis growth experiments. Although there are major differences between Arabidopsis and the cereals, Arabidopsis has in the past, and will continue into the future, to provide us with an important understanding of the NO_3^- uptake system in plants.

POS-WED-326

ARABIDOPSIS DETECTIVES: INNOVATIVE APPROACH TO RESEARCH-LED DRIVEN TEACHING

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The next generation of plant science graduates will need creativity backed by high quality knowledge and investigative skills if they are to tackle the challenges of food production and biodiversity management in the face of climate change. *Plants: Genes to Environment* (BIOL2121) is the key course introducing plant science to undergraduates at the Australian National University. This research-led course features an interactive approach by research-active staff, and innovations such as peer-assisted learning in lectures, the inquiry-based identification of *Arabidopsis thaliana* mutants in practical classes, the support of previous year students as Peer Mentors, and engaging research-based approaches to assessment. Arabidopsis is a powerful species with which to teach the basic principles of plant physiology and genetics because of the comprehensive understanding of its physiology and genetics, and an extensive collection of mutants and protocols. In the Plant Detectives project, teams of students put into practice their newly acquired theoretical knowledge as they apply cutting-edge laboratory techniques to identify Arabidopsis mutants. In describing the award-winning course's innovative design and multiple positive outcomes, we shall show how we believe the future of plant science research lies in engaging today's students as researchers and how one small plant is helping us do this.

POS-TUE-327

TEACHING TOOLS IN PLANT BIOLOGY, TO INSPIRE THE NEXT GENERATION

Williams M.E.
American Society of Plant Biologists, The Plant Cell.

Teaching Tools in Plant Biology is an online-only educational feature of *The Plant Cell*, published by the American Society of Plant Biologists. *Teaching Tools* are designed to bridge the gap between textbooks and the research literature, by providing up-to-date, hyperlinked, online content. Each *Teaching Tool* covers a key topic in plant biology through (i) a review article written for undergraduates with links to primary literature and review articles, (ii) PowerPoint slides that provide both introductory and advanced material arranged into a coherent narrative, and (iii) a teaching guide that includes learning objectives and questions for discussion or assessment. Ultimately, *Teaching Tools in Plant Biology* will cover all the major topics in plant biology at a level suitable for advanced undergraduates. A set of ten lectures covers each of the major plant hormones, and another set of eight lectures examines the biotic interactions of plants. Lectures on the theme of plant physiology will be published during 2013 and will include *Teaching Tools* on water uptake and movement, mineral assimilation, photosynthesis, and so forth. Periodically we publish *Tools* on topics of broad societal interest, such as human nutrition, medicinal plants, and biofuels. *Teaching Tools in Plant Biology* is available by institutional or personal subscription to *The Plant Cell* and to ASPB members; a pay-per-view option has just been added. Access to the first six Teaching Tools, including one that asks, '*Why Study Plants?*' is unrestricted. Have a look and tell us what you think, and please inform your students and colleagues about this innovative educational resource.

POS-WED-328

PHOTOPERIODIC FLOWERING REGULATORS INDIRECTLY AFFECT LIGHT-INDUCED STOMATAL OPENING

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Our previous study indicated that Arabidopsis florigen gene, *FLOWERING LOCUS T* (*FT*) is expressed in stomatal guard cells and has a positive effect on blue light-induced stomatal opening induced by phototropins (phot1 and phot2) probably via transcriptional regulation of downstream genes. *TWIN SISTER OF FT* (*TSF*) is the closest known homolog of *FT* and regulates photoperiodic flowering redundantly with FT. Transcriptions of *FT* and prod by transcriptional factor *CONSTANS* (*CO*) and expression of *CO* is regulated by circadian clock component *GIGANTEA* (*GI*). Here, we show that *TSF* is also transcribed in guard cells and provide a positive effect on light-induced stomatal opening. Light-induced stomatal opening was suppressed in *TSF* mutant (*tsf-1*) and *TSF*-overexpressing plants showed constitutive open-stomata phenotype. Besides, upstream regulator genes *GI* and *CO* are also transcribed in guard cells and involved in stomatal opening with accompanying changes in the transcription of both *FT* and *TSF*. Moreover, we studied about the involvement of cryptochromes in stomatal opening as photoreceptors in photoperiodic pathway. In a *cry1 cry2* double mutant, *FT* and *TSF* transcript levels decreased and light-induced stomatal opening was suppressed. Meanwhile, overexpression of *CRY2* gave increased *FT* and *TSF* transcript levels, and the stomata of the *CRY2*-overexpressing plants opened even in the dark. With respect to this, we found that *phot1 phot2* double mutant completely lacks blue light response, indicating that phototropins, but not cryptochromes, directly induce a fast stomatal response to blue light. From these results, we propose that a similar mechanism to that of photoperiodic flowering indirectly affects phototropin-mediated light-induced stomatal opening.

POS-TUE-329

CHARACTERIZATION OF ARABIDOPSIS GH5 ENZYMES

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The large and diverse glycoside hydrolase family 5 (GH5) was recently divided into 51 subfamilies. Higher plants are represented in three subfamilies: GH5_7, GH5_11, and GH5_14. Plant GH5_7 enzymes have been reported to be endo-β-mannanases (EC 3.2.1.78), but mannan transglycosylase activity has also been claimed for a few plant enzymes. The only characterized enzyme in subfamily GH5_14 exhibits β-1,3-glucosidase activity (EC 3.2.1.58), whereas subfamily GH5_11 is lacking experimental characterization. Notably, GH5_11 and GH5_14 enzymes contain putative carbohydrate-binding modules (CBMs) appended to the catalytic GH5 module and subfamily GH5_14 is not represented in Arabidopsis. Gene expression data indicates a role for plant GH5 enzymes in flower, seed, fruit and fiber development, but the function and mode of action of these enzymes are not well understood. Comprehensive biochemical characterization of recombinant Arabidopsis GH5 proteins expressed in heterologous systems will be presented.

POS-WED-330

GLOBAL PLANT COUNCIL

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The Global Plant Council (GPC) is a coalition of plant and crop science societies from across the globe. GPC seeks to bring plant scientists together to work synergistically toward solving the pressing problems we face. The central focus of the GPC is to define and engage in coordinated strategies that impact the most critical global issues; world hunger, energy, climate change, health and well-being, sustainability and environmental protection. To address these issues GPC is pursuing a series of challenges including; The Global Digital Seed Bank, Biofortification of Crops, Diversity and Yield Stability, Sustainable adaptation to changing environments. By working together to formulate a shared vision and allowing distribution of effort the GPC aims to 1. Increase awareness of the central importance of plant science, 2. Accelerate progress in solving pressing global problems via plant science based approaches 3.Enable more effective use of knowledge and resources 4.Provide a focus and contact point for plant science across the globe 5.Facilitate new research programs to address challenges. The GPC is currently made up of over 20 member organisations spread across the globe in Africa, Asia, Europe, North and South America. If your organisation would like to become a member, or if you would like to learn more about the GPC and its activities please come along to our poster or visit our website http://globalplantcouncil.org/.

POS-TUE-331

INTRACELLULAR DISTRIBUTION OF 3'-PHOSPHOADENOSINE 5'-PHOSPHATE (PAP) IN *A. THALIANA* MUTANTS AFFECTED IN PAP METABOLISM AND TRANSPORT

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The sulfation byproduct 3'-phosphoadenosine 5'-phosphate (PAP) has recently been established as a putative retrograde signalling molecule. Unless it's degraded to AMP by the respective phosphatase FRY1(SAL1) in chloroplasts or mitochondria, PAP can enter the nucleus and alter gene expression via inhibiting 5′–3′ exoribonucleases. Under various stress conditions, like drought and high-light, PAP accumulates in plants. However, the intracellular concentrations of PAP could not be measured till now, most probably, due to absence of techniques allowing PAP detection in plant extracts. In addition, the transport of PAP between cytosol and chloroplasts seems to be regulated by PAP/PAPS antiporter 1 (PAPST1) and 2 (PAPST2), representing the additional modulators of PAP signalling. In this regard, the analysis of PAP distribution in cells of *fry1*(*sal1*) but also of *fry1papst1* and *fyr1papst2* mutants is going to provide an alternative tool for understanding the role of these transporters in retrograde signalling. To address intercellular distribution of PAP within the plant cell, we used a recently described method for non-aqueous fractionation (NAF) of plant extracts (Krueger *et al*., 2010 and Klie *et al*., 2011) followed by HPLC analysis of nucleotides of interest. This approach, known also as "compartmentalized metabolome", enabled us to measure the levels of PAP and other nucleotides like PAPS, ATP, ADP and AMP in different cell compartments. Details on the distribution of these nucleotides in cytosol, chloroplast and vacuole of wild-type plants and mutants affected in PAP metabolism will be presented and discussed.

POS-WED-332

THE GENES INVOLVED IN N-GLYCOSYLATION PATHWAY STT3A AND CGL1 AFFECT THE RESISTANCE AGAINST BACTERIAL PATHOGEN PSEUDOMONAS SYRINGAE PV. TOMATO DC3000

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Protein N-glycosylation in the endoplasmic reticulum (ER) and Golgi apparatus is an essential process in eukaryotic cells. Although the N-glycosylation pathway has been shown to regulate protein quality control, salt tolerance, and cellulose biosynthesis in plants, no direct evidence showing plant immunity related roles linked functionally to N-glycan modifications that occur in the ER and Golgi apparatus was reported so far. Herein, we provide evidence that mutants defective in N-glycosylation, such as oligosaccharyltransferase mutant, staurosporin and temperature sensitive 3a (stt3a) and complex glycan 1 (cgl1), are more susceptible against bacterial pathogen Pseudomonas syringae pv tomato DC3000 than wild type Arabidopsis thaliana. The mutation in STT3a or CGL1 made plant also more susceptible to Erwinia carotovora subsp. carotovora, which cause bacterial soft rot, one of the devastating disease in Korean cabbage (Brassica rapa subsp. pekinensis).

POS-TUE-333

EARLY ENDOSOMAL COMPONENTS ARE REQUIRED FOR POLAR PIN PROTEIN LOCALIZATION AND PLANT ARCHITECTURE IN *ARABIDOPSIS*

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PIN-FORMED (PIN) family of auxin transporters are known to localize at specific sides of cells and export auxin from the cells, enabling the directional transport of auxin in the tissues. PIN proteins are rapidly shuttling between the plasma membrane and intracellular compartments, potentially allowing dynamic changes of the asymmetric localization according to developmental and environmental cues. However, information on the molecular components involved in endocytic trafficking remains scarce. By genetic and pharmacological inhibition of early endosomal trafficking, we revealed that early endosomal protein ARF GEF BEN1 is involved in early endosomal trafficking of PIN proteins. We also discovered that another mutation in the Sec1-Munc18 family protein BEN2/AtVPS45 abolishes its own early endosomal localization and compromises intracellular trafficking of PIN proteins. BEN1 and BEN2 are collectively required for polar PIN localization, their dynamic repolarization and consequently for auxin activity gradient formation and auxin-related developmental processes including embryonic patterning, root growth, organogenesis and vasculature venation patterning. These results show that early endosomal trafficking is crucial for cell polarity and auxin-dependent regulation of plant architecture.

POS-WED-334

BRASSINOSTEROID-MEDIATED INCREASE IN CROP YIELD AND RESISTANCE TO ABIOTIC AND BIOTIC STRESSES

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Brassinosteroids (BRs) are a group of naturally occurring plant steroidal compounds with wide ranging biological activity. Because BRs control important agronomic traits such as plant architecture, biomass, seed yield and stress tolerance, the genetic manipulation of BR pathways and BR-related genes offers a unique possibility of significantly increasing crop yields through changing plant metabolism and protecting plants from environmental stresses. In continuing our work on understanding the molecular mechanisms underlying BR-mediated increase in stress tolerance, we analyzed global gene expression in unstressed and stressed BR-treated and untreated *Arabidopsis thaliana* seedlings using ATH1 microarrays. An initial analysis of T-DNA mutants of a subset of genes allowed us to identify new genes related to yield and stress tolerance. Detailed analysis of a few genes was carried out in Arabidopsis through studying phenotypes of loss-of-function mutants and overexpressor lines, subcellular localization and protein:protein interactions. The phenotypes associated with these alleles include increased branching, early flowering, increased seed yield (20-40%) and increased tolerance to abiotic stresses. Further, we determined stress tolerance-related traits in transgenic *Brassica napus* lines expressing a BR biosynthesis gene *AtDWF4* under the control of the CAMV 35S promoter. The transgenic lines, in addition to increased vegetative growth and seed yield, showed enhanced tolerance to heat and dehydration stress, as well as enhanced resistance against two fungal pathogens, which correlated with significantly higher expression of several defense-related genes in transgenic lines as compared with wild type plants. These results confirm the value of research on BRs to help meet the goals of productivity, sustainability and environmental benefits, and provide insight into BR-related mechanisms associated with yield and stress tolerance.

POS-TUE-335

EVOLUTIONARY CONSERVED MOTIFS INVOLVED IN ACTIVITY AND REGULATION OF THE ABA-INSENSITIVE (ABI) 4 TRANSCRIPTION FACTOR

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The transcription factor ABI4 is an important integrator of multiple signals including nutrient status, stress responses, and hormone signaling. The integration of these signals is particularly critical during some developmental transitions of the plant. However, the almost negligible levels of the ABI4 protein in plants under the different conditions analyzed has significantly limit understanding the mechanism of action and regulation of this protein. To better understand the function and regulation of ABI4 we performed a functional analysis of evolutionary conserved motifs in this protein. The functionality of the putative ortholog from *Theobroma cacao*, using transient expression assays and complementation studies, support that these selected motifs are good identifiers of ABI4 orthologs in different plants species. The function of these conserved motifs was analyzed through deletion or mutagenesis in the Arabidopsis ABI4 protein. Due to difficulty of a direct analysis of the protein in transgenic plants in this work an alternative approach using transient expression assays in Arabidopsis mesophyll protoplast was taken. This approach permitted to detect immunologically the ABI4 protein and identify some of the mechanisms involved in the regulation of this protein. We identified specific motifs that are required for the nuclear localization and for the transcriptional activation function of this factor. This approach also permitted to demonstrate that the protein stability of this transcription factor is controlled through multiple mechanisms that involved the PEST motif and the 26S proteasome.

POS-WED-336

COMPARATIVE TRANSCRIPTOME ANALYSIS OF ENERGY-RICH *ARABIDOPSIS THALIANA* UNDER DARK AND LIGHT CONDITIONS

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Overexpression of Arabidopsis thaliana purple acid phosphatases AtPAP2 in Arabidopsis can promote plant growth. The overexpression (OE) lines flower earlier, grow faster, and contain more ATP, sucrose and glucose contents. The seed yield and silique numbers of OE lines are also more than the control lines (Sun et al., 2012). In this study, we compared the leaf transcriptomes of 20-d-old transgenic and wild-type Arabidopsis grown under long day (16h/8h) condition. Total RNA were collected at three time points: end of night (t=0 hr), one hour after light was turned on (t=1 hr), and eight hours after light was turned on (t=8 hr). AtPAP2 is dually targeted to chloroplasts and mitochondria. To study the RNA encoded by chloroplasts and mitochondria, ribosomal RNAs were removed before sequencing. Approximate 65 million clean reads (~6Gbp) were obtained by Illumina HiSeqTM 2000 sequencing from each library. In total, after assembly 29,435 transcripts are generated from the six libraries. More genes are suppressed in OE leaves (vs WT) at all the three time points, 1,623 (down-regulated) versus 945 (up-regulated), 1,908 (down-regulated) versus 712 (up-regulated) and 1,642 (down-regulated) versus 824 (up-regulated) at t=0, 1 and 8 hours, respectively. Expression profiles of light-induced transcripts based on K-means clustering were analyzed. There are significant changes in the transcription levels of various components of photosystems, light-harvesting chlorophyll protein complexes (LHC), respiratory complexes, RNA polymerases and ribosomes. Our data provide systemic portraits of global changes in Arabidopsis transcriptome exerted by dark to light transition (external energy input) and high internal energy status.

POS-TUE-337

HSP90 MEDIATES TEMPERATURE ENTRAINMENT OF THE CIRCADIAN CLOCK

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Arabidopsis has evolved an internal-timing mechanism to respond to predictable environmental changes and these include daily temperature fluctuations. This timing system has been termed the circadian clock. The mechanism of temperature setting of the plant clock has remained unclear. Since unpredictable fluctuations in temperature may cause structure alterations of clock components, and as heat shock protein (HSP) 90 serves as a protein chaperon that helps stabilization of its client proteins, we examined if HSP90 is a component for temperature regulation in the circadian clock. For this, we measured clock speed in an hsp90 mutant line and found it displayed a longer period specifically after the clock was set by temperature. From this, we applied the HSP90 inhibitor geldanamycin (GDA) to plants to phenocopy the mutational effect. In GDA treatment assays, the HSP90 effect on the oscillator was targeted to specific clock genes. In addition, our work revealed that HSP90 is involved in supporting the temperature-dependent transcription of two clock genes, and this explained thermal resetting. An in vivo and in vitro protein-binding assay was performed and HSP90 was found to physically interact with several clock components. Together, this has given us a good starting point to analyze how HSP90 mediates temperature sensing to reset the clock.

POS-WED-338

A RETROTRANSPOSITIONAL REGULATION OF A HEAT-ACTIVATED RETROTRANSPOSON

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Transposable elements (TEs) constitute a large fraction of most eukaryote genomes that can insert into new chromosomal locations. However, most of the TEs are silenced because of epigenetic regulations such as DNA methylation and histone modification. In plants, small interfering RNAs (siRNAs) derived from RNA polymerase IV (polIV) is required for RNA directed DNA methylation and TEs silencing. In recent report, a copia-type retrotransposon named *ONSEN* was activated by heat stressed in *Arabidopsis thaliana. ONSEN* expression was much higher in mutants impaired in polIV compared to wild-type. Surprisingly, high frequency of retrotransposition was detected in the progenies of heat-stressed siRNA mutant plants. To clarify a mechanism of the retrotransposition, we examined the frequency of a transpositional event in various developmental stages and stress conditions. *ONSEN* retrotransposition pattern suggested that there would be tissue specific regulation.

POS-TUE-339

THE SINGLE-STRANDED DNA BINDING PROTEIN *WHIRLY1* REPRESSES *WRKY53* EXPRESSION AND LEAF SENESCENCE IN *ARABIDOPSIS THALIANA*, IN A DEVELOPMENTAL STAGE-DEPENDENT MANNER

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Leaf senescence in plants involves sophistical transcriptional regulations, both positively and negatively. Here we showed evidence for single-stranded DNA (ssDNA) binding protein WHIRLY1 function as an upstream suppressor of leaf senescence in Arabidopsis. *WHIRLY1* mutants or its antisense plants (*why1* or *awhy1*) displayed an early senescence phenotype, and increased transcript levels of both the senescence regulator *WRKY53* and the senescence associated protease gene *SAG12*. WHIRLY1 bound to the conserved sequence motif *GNNNAAATT* plus *AAAT* of promoter of *WRKY53* in an *in vitro* mutagenesis assay as well as in a chromatin immunoprecipitation (ChIP) assay. This direct interaction was further confirmed by a transient expression assay in which WHIRLY1 repressed *WRKY53* promoter driven gene expression. Genetic analysis of double-mutants transgenetic plants revealed that neither WHIRLY1 overexpression (*oeWHY1 wrky53*) nor knock-out (*why1 wrky53*) had an effect on the stay-green phenotype of *wrky53*. Taken together, these results suggested that WHIRLY1 was an upstream regulator of WRKY53 during leaf senescence. This regulation was developmental-stage dependent, as further verified by a ChIP-PCR analysis.

POS-WED-340

PLANT RECEPTOR KINASES AS TARGETS FOR BACTERIAL EFFECTORS

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Pathogenic bacteria have evolved multiple ways to facilitate the infection of their respective host plants. Co-evolution of plant defense mechanisms has led to an evolutionary arms race where secreted bacterial proteins, termed effectors, target various plant proteins to suppress plant innate immunity. In turn, these effectors can be recognized by specialized plant resistance proteins. The bacterium *Pseudomonas syringae* pv. tomato, a well studied natural pathogen of tomato, can use a battery of effector proteins to aid in its virulence. One of these effectors, termed AvrPto, has in previous studies been described as an interactor of various receptor-like kinases in the model plant *Arabidopsis thaliana*. Among these are FLS2 (FLAGELLIN SENSING 2), the plant flagellin receptor, as well as BAK1 (BRI1-ASSOCIATED KINASE 1), a protein with multiple roles including innate immunity and brassinosteroid signaling. Currently, there is no consensus about which plant protein is the naturally favored target of AvrPto. To aid in understanding the biochemistry of this bacterial effector, interaction studies including AvrPto as well as various heterologously expressed and purified kinase domains of *A. thaliana* receptor-like kinases will be performed. Determination of quantitative binding affinities should give an idea about which part of the complex innate immunity signalling cascade is of special importance for bacterial virulence.

POS-TUE-341

ARABIDOPSIS AMIDASE1 CONTRIBUTES TO AUXIN BIOSYNTHESIS *IN VIVO*

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Plant growth and development largely relies on the plant hormone indole-3-acetic acid (IAA), the most abundant naturally occurring auxin. Despite of numerous decades of investigation, auxin biosynthesis is yet not entirely elucidated. However, just recently remarkable progress has been made towards its disclosure. At present, auxin formation is assumed to proceed via very few biosynthetic pathways, but their crosstalk has yet to be completely defined. Amidases capable of converting indole-3-acetamide (IAM) into IAA are suggested to contribute to auxin biosynthesis. We have characterized three allelic *Arabidopsis thaliana ami1* (*amidase1*) mutants, who share defects in an IAM-specific hydrolase, a homolog of the auxin synthetic iaaH gene from Agrobacterium. The *ami1* mutants have lower IAM hydrolase activities and reduced levels of IAA. In addition, they show a subtle root phenotype that becomes more pronounced under stress conditions. *AMI1* is predominantly expressed in proliferating tissues throughout plant development. The overexpression of *AMI1* led to a significant alteration of root development, curled leaf growth, and premature establishment of reproductive organs. The overexpressors displayed higher amidase activities and increased auxin contents. Furthermore, we quantitatively analyzed *AMI1* transcription levels in numerous auxin biosynthetic mutants. The obtained results point towards sophisticated relationships between the pathways. As we were able to isolate and characterize AMI1-homologs from various plant species, we conclude that IAM hydrolase-dependent IAA biosynthesis is a widely distributed concept in the plant kingdom.

POS-WED-342

EFFECT OF PARENTAL REPRODUCTIVE AGE ON THE SPONTANEOUS MUTATION RATES IN *ARABIDOPSIS*

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Abstract: Little is known on the effect of parental reproductive age on the somatic mutation rates in flowering plants. With a set of *Arabidopsis* mutation detector lines we examined the effect of parental reproductive age on the somatic mutation rates in the progeny. We scored point, frameshift, homologous recombination and transposition rates based on functional GUS reversion of a mutated or truncated uid A gene. We observed that T-C transition rates increased with the parental reproductive age in the offsprings. Similarly, frameshift mutation (G10) and transposition rates also rise in the progenies as a consequence of increased parental age and the effect is pronounced with the increased maternal age. However, homologous recombination frequency decreased with the age of parents. Our study shows that parental reproductive age alters the somatic mutation rates in progeny plants of *Arabidopsis* similar to what is known in humans.

POS-TUE-343

AN ORTHOLOG OF HUMAN TRAF-LIKE PROTEIN OF *ARABIDOPSIS THALIANA* IS ESSENTIAL FOR POLLEN WALL DEVELOPMENT

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The tumor necrosis factor (TNF) receptor-associated factors (TRAFs) serve as adapter proteins for a wide variety of cell surface receptors and have several roles, which are both unique and overlapping. These include activation of kinases and transcription factors, and interactions with other signaling proteins, culminating in the induction or inhibition of biological functions such as cell survival, apoptosis, and cell differentiation. Till date, the role of TRAFs in reproductive development has not been elucidated in plants. Screening of promoter trap population identified a line that showed abnormal pollen. The T-DNA insertion was found in TRAF-like gene, which is an ortholog of Human TRAF. Analysis of total lipids of pollen revealed that the mutant pollen were deficient in long chain fatty acids. The results indicate that TRAF plays an important role in pollen wall development. Development and maturation pollen involves a number of signaling cascades and several adaptor-mediated processes which orchestrate diverse processes such as tapetum differentiation, programmed cell death, pollen wall formation and release of pollen from the anthers.

POS-WED-344

ATML1 PROMOTES EPIDERMAL CELL DIFFERENTIATION IN ARABIDOPSIS SHOOTS

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Molecular mechanisms that generate distinct tissue layers in plant shoots are not well understood. Cell-type specific transcription factors play key roles in determining cell fate through the regulation of gene expression. *ATML1*, an Arabidopsis homeobox gene, is expressed in the outermost cell layer beginning at an early stage of development. The promoters of many epidermis-specific genes, including *ATML1*, contain an ATML1-binding site called an L1 box, suggesting that ATML1 regulates epidermal cell fate. However, whether *ATML1* is sufficient for the activation of these epidermis-specific genes and for the induction of epidermal cell fate in non-epidermal cells is unknown. Here, we show that overexpression of *ATML1* was sufficient to activate the expression of epidermal genes and to induce epidermis-related traits such as the formation of stomatal guard cells and trichome-like cells in non-epidermal seedling tissues. These results support the idea that ATML1 acts as a master regulator of epidermis differentiation. Detailed observation of the division planes of these ectopic stomatal cells suggested that a near-surface position as well as epidermal cell identity were required for regular anticlinal cell division, as seen in wild-type epidermis. Moreover, analyses of a loss-of-function mutant and overexpressors implied that differentiation of epidermal cells was associated with repression of mesophyll cell fate. Collectively, our studies contribute new information about the molecular basis of cell fate determination in different layers of plant aerial organs.

POS-TUE-345

ARABIDOPSIS CRINKLY 4 IS A DOWNSTREAM TARGET OF ATML1-MEDIATED TRANSCRIPTIONAL REGULATION

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The plant epidermis is a single layer of the cells, which protect plants from environmental stress and pathogen attack. The *Arabidopsis ATML1* gene, a member of the HD-GL2 class of homeobox genes, plays a key role in the differentiation of shoot epidermal cells. It has been proposed that ATML1 promotes expression of epidermis-specific genes through the direct binding to L1 box sequences in the promoters of its target genes. However, little is known about the direct targets of ATML1.In this study, we focused on the *ARABIDOPSIS CRINKLY4 (ACR4)* gene as a candidate that regulates epidermal cell differentiation downstream of ATML1. *ACR4* encodes an epidermis-specific receptor-like kinase that localizes to the plasma membrane of epidermis. Loss-of-function mutations in *ACR4* have been shown to slightly affect the differentiation of epidermis. Our expression studies showed that *ACR4* mRNA increased in *ATML1*-overexpressing plants while decreased in *atml1;pdf2*. Moreover, we found two L1-box like sequences in the *ACR4* promoter. Mutations in one of the L1-box like sequences decreased the activity of the *ACR4* promoter. Further, chromatin immunoprecipitation analysis localized ATML1 to the L1-box like sequence of *ACR4*. Together, these results suggested that ATML1 directly binds to the *ACR4* promoter and positively regulates *ACR4* expression. Possible roles of *ACR4*, as a downstream effector of ATML1, will be discussed.

POS-WED-346

ENTRAINMENT THROUGH THE ARABIDOPSIS CIRCADIAN CLOCK FACTOR *ELF3* IS CONVEYED BY CELLULAR LOCALIZATION, AS REVEALED BY NATURAL VARIATION STUDIES OF AN ALTITUDE-ASSOCIATED ALLELE

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Genomes interact with the environment and give rise to variations in traits, some of which survive and evolve into unique populations. Natural selection of allelic variants within the Arabidopsis thaliana circadian clock can be attributed to adaptation to varying environmental conditions. More specifically, natural selection acts to maintain suitable combinations of clock alleles that provide fitness advantages under given habitats. To define the molecular origin of such allelic variation in the clock, we examined clock speed in a reporter-modified Bay-0 x Shakdara recombinant inbred line and localized heritable variation. Extensive variation led us to identify a major quantitative trait locus (QTL) that was fine-mapped to *EARLY FLOWERING3* (*ELF3*). The causal polymorphism in the *ELF3-Sha* allele was an encoded amino-acid replacement, which caused a short-period phenotype under light and severely dampened rhythm generation in darkness. Circadian oscillations in *ELF3-Sha*-harboring lines could not be properly reset by light. We found that ELF3-Sha protein failed to properly localize to the nucleus, and its ability to accumulate in darkness was compromised. We performed population genetic analysis on the *ELF3* locus and provided evidence that the *ELF3-Sha* allele is associated with higher altitudes in Central Asian accessions. Collectively, by characterizing *ELF3-Sha*, we showed that ELF3 protein plays a vital role in defining its light-repressor function in the circadian clock and that this is a process under the constraints of natural selection.

POS-TUE-347

EMERGING EVIDENCE ON THE ROLE OF CELL WALL INVERTASE IN REGULATING VASCULAR DEVELOPMENT

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Vascular development generates xylem and phloem, which constitute a continuous cellular network essential for mechanical support and transport of resources and signaling molecules throughout the plant body. Significant progresses have been made in understanding the differentiation and pattern formation of vascular tissues, which largely focused on roles of transcriptional factors, microRNAs, and signaling molecules, such as auxin, cytokinins in vascular development. Here, we present data to show that cell wall invertase (CWIN) could act as a novel player in regulating vascular development based on analyses of Arabidopsis CWIN gene T-DNA insertion mutants. In comparison with wild type, *AtCWIN1* single mutant (*cwin1*) and *AtCWIN2* and *AtCWIN4* double mutant (*cwin2*4*) exhibited thinner inflorescence stems. The phenotype is caused by significantly reduced xylem cell layers and smaller pith. It is worth noting that despite the similar phenotype of *cwin1* and *cwin2*4*, the underlying biological mechanism are probably different, as the former may be caused by an effect on vascular differentiation during inflorescence stem development, while the latter is likely to be a consequence of an impact on provascular development during embryogenesis (Wang and Ruan, 2012, Plant Physiol 160, 777-787). These findings indicate novel roles CWIN could play in vascular development both during and post embryogenesis.

POS-WED-348

FUNCTIONAL CHARACTERIZATION OF NON-CYTOLYTIC MEMBERS WITHIN THE NLP EFFECTOR SUPERFAMILY

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NEP1-like proteins (NLPs) represent a superfamily of effector proteins, which are distributed amongst various oomycetes, fungi and bacteria. Among the best-characterized NLPs are those that are expressed and secreted by the pathogens during the late stage of infection and that trigger necrosis in dicotyledonous plants. This necrotic activity makes them virulence factors in the host organisms by supporting a necrotrophic and hemibiotrophic life style. The three-dimensional structure of an NLP from *Pythium aphanidermatum* (NLP_{Pya}) is similar to the fold of actinoporins, pore-forming toxins from marine invertebrates. Like actinoporins, NLPs affect the integrity of plasma membranes from dicot plants, thereby leading to necrosis. Besides cytolytic NLPs, another group of NLPs exists which is expressed in the early infection stage. High numbers thereof are present in oomycetes and fungi (e.g. in *Phytophthora infestans* and *Verticilium dahliae)*, and are found in biotrophic organisms like the Arabidopsis pathogen *Hyaloperonospora arabidopsidis*. As expected due to the lack of conserved residues required for cytolysis, these NLPs are incapable of triggering cytolysis and necrosis. Considering the structural relation of NLPs also to fungal lectins, non-cytolytic NLPs could mediate host cell adhesion and thereby contribute to virulence in the early infection process. Using heterologously expressed protein, the membrane binding behavior of non-cytolytic NLPs was examined and differences to cytolytic NLPs shall be analyzed in a crystallization approach. To study the importance of non-cytolytic NLPs in plant pathogen interactions, selected members were studied in regard to their effect on plant infection experiments, being either silenced or overexpressed.

POS-TUE-349

CYTOLYTIC TOXINS OF THE NEP1-LIKE PROTEIN FAMILY RELEASE DAMPS AS MOBILE SIGNAL OF DANGER

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The activation of innate defense mechanisms of plants against microbial infection is mainly based on two branches: the PRR-mediated recognition of PAMPs (pathogen-associated molecular patterns), called PAMP-triggered immunity and the recognition of microbial effectors by receptors encoded by R-genes, called effector- triggered immunity. Besides these two mechanisms of pathogen perception, plants can also sense endogenous patterns, representing stress-associated molecules (damage-associated molecular patterns, DAMPs), which induce innate immunity. Such endogenous elicitors so far comprise cell wall fragments, cutin monomers and peptides like systemin and AtPEP1. Well-known triggers of plant immune responses are necrosis and ethylene-inducing peptide 1 –like proteins (NLPs). NLPs are virulence-promoting toxins found in phytopathogenic bacteria, oomycetes and fungi. By disrupting the plasma membrane of dicotyledonous plants, NLPs are inducing cell death and thus contribute to the virulence of necrotrophic and hemibiotrophic plant pathogens. The mechanism of membrane disruption might be similar to that of structurally related pore-forming toxins from marine invertebrates, but remains to be elucidated. Studies with active and inactive mutant versions of the NLP from the oomycete Pectobacterium carotovorum showed, that not the NLP molecule itself is recognized, but its membrane disrupting activity. Thus, it is very likely that the activity of NLPs induces the production of breakdown products or the release of intracellular molecules that are sensed as DAMPs. The identification of those plant-derived DAMPs and their corresponding receptors will help to elucidate this novel form of plant innate immunity.

POS-WED-350

LYSIN-MOTIF-PROTEINS MEDIATE PEPTIDOGLYCAN-PERCEPTION IN *ARABIDOPSIS THALIANA*

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Recognition of microbial patterns by host pattern recognition receptors is a key step in immune activation in multicellular eukaryotes. Peptidoglycans (PGN) are major components of bacterial cell walls that possess immunity-stimulating activities in metazoans and plants (1). Recently, we could show that PGN sensing and immunity to bacterial infection in Arabidopsis thaliana requires three lysin-motif (LysM) domain proteins (2). LYM1 and LYM3 are plasma membrane proteins that physically interact with PGN and that mediate peptidoglycans responses. lym1 and lym3 mutants lack PGN-induced changes in transcriptome activity patterns, but respond to fungus-derived chitin, a pattern structurally related to PGN, in a wild-type manner. Notably, lym1, lym3 and lym1 lym3 mutant genotypes exhibit super-susceptibility to infection with virulent Pseudomonas syringae pathovar tomato (Pto) DC3000. Defects in basal immunity in lym1 lym3 double mutants resemble those observed in lym1 and lym3 single mutants, suggesting that both proteins are part of the same recognition system. We further show that deletion of CERK1, a LysM receptor kinase that had previously been implicated in chitin perception and immunity to fungal infection in Arabidopsis, phenocopies defects observed in lym1 and lym3 mutants, such as peptidoglycan insensitivity and enhanced susceptibility to bacterial infection. Altogether, we show that Arabidopsis harbours a bipartite PGN recognition system comprised of LYM1/LYM3 for PGN ligand binding and CERK1 that is likely required for conveying the extracellular signal across the plasma membrane and for initiating intracellular signal transduction. Future work will now focus on a potential physical interaction of these proteins.

POS-TUE-351

FUNCTIONAL ROLES OF CELL WALL POLYSACCHARIDES DURING OVULE AND SEED DEVELOPMENT

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The composition of polysaccharides within the plant cell wall varies greatly depending on the species, developmental stage, external stimuli and the identity of the organ. Dynamic alterations in the amount and structure of plant cell wall polysaccharides are particularly evident during ovule and seed development. Despite this, little is known about the biosynthetic and hydrolytic enzymes that act on a tissue and cell-specific level in female reproductive tissues to influence cell identity, function and fate. Using Arabidopsis, Plantago and barley as model systems, we are generating histochemical and transcriptional profiles of specific developing tissues to characterise polysaccharide composition and cell wall-related gene expression. This has led to the identification of genes encoding putative biosynthetic and hydrolytic enzymes, for which the role of the corresponding polysaccharides in development is unknown. Interestingly, inter- and intraspecific variation in polysaccharide composition between species correlates with changes in the biosynthetic machinery. The functional significance of these differences is currently being investigated.

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