FULL ABSTRACTS LIST

Note: ABSTRACT NUMBERING AND POSTER SESSION SCHEDULE

All submitted abstracts have been renumbered from the original submission assignment. Abstracts #1-
#52 are from invited speakers. Abstracts numbered #53 and higher are from general participant
submission and grouped according to the session to which they were submitted.

Abstracts numbered #53 and higher will have one of three designations next to them:
• Poster: The abstract is presented as a poster only
• Talk: The abstract is presented in an oral presentation only, in a concurrent session or workshop
• Poster/Talk: The abstract is presented as a talk (in a concurrent session or workshop) AND as a
  poster
• [The original abstract Submission number assigned by the abstract database follows each abstract
title]

There will be two official Poster Sessions, one Tuesday evening and one Thursday evening. To
determine when you should stand next to your poster, find your abstract in this list by scanning the
session that you submitted your abstract to. Note the new abstract number. The new number is your
poster number (if you are presenting a poster) and it is also your oral presentation number (if you are
giving an oral presentation.) These new numbers are listed in the printed program next to talks.

All posters with EVEN numbers will be presented on Tuesday evening from 6:15-7:15 pm.
All posters with ODD numbers will be presented on Thursday evening from 6:15-7:15 pm.

Note: Poster and exhibit sessions open at 5 pm and close at 8 pm.
Open browsing of all Posters is encouraged before and after the designated presentation times
listed above.

Invited Speakers

Keynote Invited Speaker #1. Understanding plant-microbe interactions: Plant immune system function
and the rhizosphere microbiome (Submission 289)
Jeff Dangl

1HHMI and Univ. of North Carolina at Chapel Hill, United States

The plant immune system is a sophisticated two-tiered receptor-based system that utilizes transmembrane
pattern recognition receptors to survey microbe associated molecular patterns (MAMPs). Recognition mediated
by this ‘first tier’ of the plant immune system generates a set of downstream responses that are sufficient to stop
the growth of most microbes, termed MTI (MAMP-triggered immunity). Pathogens, by definition, can surpass or
evade MTI and they do so by deploying into the host cell suites of virulence effectors. Plants have therefore
evolved a second, intracellular receptor system, consisting of NLR proteins, which monitor effectors directly as ligands, or via their alteration of NLR-associated host targets. Activation of an NLR essentially 're-boots' suppressed MTI, but does so in a very rapid and high amplitude manner termed ETI (Effector-triggered immunity). Our first set of goals are to understand how particular pathogen effector proteins have evolved to manipulate host signaling machinery to function as virulence factors; how these molecular manipulations are recognized by the intracellular NLR receptors; and how NLR activation initiates a successful immune response. Our rationale is that by understanding how a broad collection of virulence factors from evolutionarily diverse pathogens act inside the host cell, we will better understand the normal defense relevant function of their targets. Our second set of goals is to understand how the microbiome colonizing the root rhizosphere and the root-intercellular endophytic compartment is organized. The root microbiome contributes to plant growth and development, crop productivity, carbon sequestration and phytoremediation. Colonization of the rhizosphere and endophyte compartment occurs in the presence of a sophisticated plant immune system, suggesting finely-tuned discrimination of pathogens from mutualists and commensals. The principles that govern the winnowing of the complex soil microbial community into host-specific rhizosphere and endophyte compartments are largely unknown. We are defining these principles via characterization of the Arabidopsis rhizosphere microbiome using synthetic microbial communities.

Keywords: Plant immune system, synthetic biology, microbiome, type III effectors, NLR proteins

Invited Keynote Speaker #2. Understanding small RNAs - from Arabidopsis to crops and humans (Submission 475)
Xuemei Chen

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Small RNAs of 20-30 nucleotides have been recognized to impact almost all biological processes as sequence-specific regulators of genes and genomes in plants and animals. Given what we now know about the fundamental roles that small RNAs play in growth, development, immunity, and disease, it might be surprising to some that the widespread presence of small RNAs has only been known for 15 years. In 1999, small interfering RNAs (siRNAs) were discovered in plants and associated with posttranscriptional gene silencing. In 2001, the widespread existence of microRNAs (miRNAs) in animals became known, followed by the discovery of miRNAs in plants in 2002. The past 15 years have been an exciting and rewarding time in small RNA biology, as research in plant and animal models simultaneously and synergistically unveils the secrets of small RNAs - their biogenesis, turnover, modes of action in transcriptional and posttranscriptional gene silencing, and the numerous biological processes that they impact. The knowledge of small RNA-based gene silencing has in turn revolutionized biological research and contributed to the development of enabling technologies in crop improvement and disease therapy.

Thanks to the superior genetic and genomic resources, at the dawn of small RNA biology, Arabidopsis was poised to be the model from which many fundamental discoveries in RNA silencing were later to be made. I will review the short but eventful history of small RNA biology with a focus on how research in Arabidopsis has led to the molecular frameworks of RNA silencing in and beyond plants. I will discuss work from my lab on the mechanisms of miRNA turnover as an example of how work in Arabidopsis could inform small RNA biology in general.

Keywords: RNA silencing, siRNA, miRNA

Invited Speaker #3. Epigenetic Inheritance of silent locus identity (Submission 337)
Craig Pikaard, Todd Blevins
Howard Hughes Medical Institute, Indiana University, United States, Indiana University, United States
Multisubunit RNA polymerases IV and V orchestrate RNA-directed DNA methylation (RdDM) and transcriptional silencing of transposons and other genomic repeats, but what identifies the loci to be silenced and provides signals for Pol IV/V recruitment is unclear. We have found that heritable silent locus identity at a specific subset of RdDM targets requires HISTONE DEACETYLASE 6(HDA6), which acts upstream of Pol IV recruitment and siRNA biogenesis. At these loci, epigenetic memory conferring silent locus identity is erased in hda6 mutants such that restoration of HDA6 activity cannot restore siRNA biogenesis or silencing. Silent locus identity is similarly lost in mutants for the cytosine maintenance methyltransferase, MET1. By contrast, pol IV or pol V mutants
To capture the dynamics of the chromatin landscape in response to two important environmental cues, light and heat shock, we treated seedlings with increasing light intervals or a short, severe heat shock. We documented thousands of changes in the cis-regulatory DNA landscape and TF networks across these conditions. Many light-activated cis-regulatory regions occurred near transcription factors and photosynthesis-related genes while dark-activated cis-regulatory regions were enriched near auxin-responsive genes. Although TF network connectivity peaked after 3hrs of light exposure, several bZIP factors, including HY5, were highly connected across all light treatments. In response to heat shock, a unique chromatin signature arose that was associated with heat-activated genes, including canonical heat-shock protein genes HSP90 and HSP101 as well as novel heat shock-

Keywords: DNA methylation, histone modification, gene silencing, chromatin, epigenetics

Invited Speaker #4. Polycomb-mediated regulation of floral stem cells (Submission 328)
Toshiro Ito

Temasek Life Sciences Laboratory, Singapore

In an indeterminate shoot apical meristem, stem cells persist for the life of the plant. In contrast, in a determinate floral meristem, a fixed number of organs are produced before the meristem is terminated. In Arabidopsis, the floral stem cell identity is sustained by expression of the homeo-domain transcription factor WUSCHEL (WUS). Expression of WUS is terminated by multiple genetic pathways, including the zinc finger protein KNUCKLES (KNU), with the result that stem cell identity is inactivated. KNU expression is induced by the floral homeotic protein AGAMOUS (AG). The induction process requires approximately two days and entails the dynamic changes of the repressive histone modification H3K27me3 at the KNU locus. The precise temporal induction of KNU is essential for the coordinated growth and differentiation of floral stem cells, but the mechanism that control this timing has remained unclear. Here we present an epigenetic mechanism in which the floral homeotic protein AG induces KNU at approximately two days of delay. AG binding sites colocalize with a Polycomb Response Element in the KNU upstream region. AG binding to the KNU promoter causes the eviction of the Polycomb group proteins (PcG) from the locus, leading to cell division-dependent loss of the repressive status. We reconstructed an epigenetic-based synthetic circuit in cell lines that shows similar delayed-induction. These analyses demonstrate that floral stem cells measure developmental timing by a division-dependent epigenetic timer triggered by PcG eviction. We also discuss how KNU represses WUS expression through epigenetic regulation and our current approach to reveal molecular details of recruitment and modification of PcG activity in floral meristems.

Keywords: Flower development, Polycomb, Floral meristems, Synthetic Biology

Invited Speaker #5. Mapping and dynamics of regulatory DNA and transcription factor networks in A. thaliana (Submission 282)
Alessandra Maria Sullivan1, Andrej Arsovski1, Janne Lempe1, Kerry L Bubb1, Matthew T Weirauch2, Peter J Sabo1, Richard Sandstrom1, Robert E Thurman1, Shane Nep1, Alex P Reynolds1, Andrew Stergach1, Benjamin Vernot1, Audra K Johnson1, Eric Haugen1, Agnieszka Dargiel1, Jun Neri1, Morgan Diesel1, Timothy R Hughes2, Jennifer L Nemhauser1, John A Stamatoyannopoulos1, Christine Queitsch1

University of Washington, United States, University of Cincinnati, United States

Our understanding of gene regulation in plants is fundamentally constrained by our limited knowledge of plant cis-regulatory DNA and its dynamics. Using a nucleotide-resolution method of cis-regulatory DNA profiling, DNase I-seq, we created a catalogue of cis-regulatory DNA, regulatory protein occupancy, and novel DNA binding motifs in the reference plant A. thaliana. We found evidence that an excess of phenotype-associated genetic variants resides within cis-regulatory DNA and that transcription factor (TF) binding preferences may drive codon bias. We leveraged our data to reveal regulatory relationships in unprecedented detail by generating empirical TF networks for A. thaliana, which shared network motif topology with other eukaryotic systems. To capture the dynamics of the chromatin landscape in response to two important environmental cues, light and heat shock, we treated seedlings with increasing light intervals or a short, severe heat shock. We documented thousands of changes in the cis-regulatory DNA landscape and TF networks across these conditions. Many light-activated cis-regulatory regions occurred near transcription factors and photosynthesis-related genes while dark-activated cis-regulatory regions were enriched near auxin-responsive genes. Although TF network connectivity peaked after 3hrs of light exposure, several bZIP factors, including HY5, were highly connected across all light treatments. In response to heat shock, a unique chromatin signature arose that was associated with heat-activated genes, including canonical heat-shock protein genes HSP90 and HSP101 as well as novel heat shock-
related genes. Although TF networks broadly lost connectivity in response to heat-shock, several heat shock transcription factors became highly connected upon heat shock. This data provides a resource for the plant community and offers novel insights into the regulation of fundamental processes of plant biology.

Keywords: TF networks, cis-regulatory DNA, Heat shock, photomorphogenesis

**Invited Speaker #6. Bacterial pathogenesis as a probe of Arabidopsis biology** (Submission 339)

Sheng-Yang He

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Despite their ability to mount powerful immune responses, plants are still highly susceptible to a variety of pathogens. Understanding why the plant immune system fails to prevent pathogen infection is a topic of substantial importance to agriculture. We have been using the Arabidopsis thaliana-Pseudomonas syringae pv. tomato (Pst) DC3000 pathosystem to understand how pathogens cause disease. Pst DC3000 produces a variety of virulence factors, including "effector proteins" that are secreted into the plant cell and the phytotoxin coronatine, which is a molecular mimic of the plant hormone jasmonate. Studies of the molecular action of Pst DC3000 effectors and coronatine have contributed to the discovery and characterization of components of innate immune signaling, jasmonate signaling, stomatal defense, and vesicle trafficking in Arabidopsis. I will present some of our new results that show diverse cellular processes targeted by Pst DC3000 effectors, the crystal structure of the JAZ/MYC complex involved in jasmonate signaling, and a potentially fundamental role of microbiota in training the Arabidopsis innate immune system. These results continue to illustrate that bacterial pathogenesis can be a useful probe of plant biology.

Keywords: biotic stress, plant immunity, pathogenesis, hormone signaling, Vesicle trafficking, stomata

**Invited Speaker #7. Fungal Small RNAs Suppress Plant Immunity by Hijacking Host RNAi Machinery** (Submission 442)

Arne Weiberg1, Ming Wang1, Feng-Mao Lin2, Hongwei Zhao1, Zhihong Zhang1, Isgouhi Kaloshian1, Hsien-Da Huang2, Hailing Jin1,* (hailingj@ucr.edu)

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Small RNAs (sRNAs) are a class of short non-coding regulators that mediate gene silencing in a sequence-specific manner. Small RNAs have been shown to play an important role in host immune responses against pathogen attacks. Most fungal genomes encode components of RNAi machinery, including Dicer-like proteins and Argonautes (AGOs). The role of fungal small RNAs in genome defense, heterochromatin formation, and gene regulation has been demonstrated. However, it was not known whether fungal small RNAs or RNAi are directly involved in pathogenicity. Botrytis cinerea is an aggressive fungal pathogen that infects more than 200 plant species. Genome-wide small RNA profiling from B. cinerea-infected Arabidopsis and tomato has identified a group of small RNAs from B. cinerea that can potentially target important regulatory genes in plant hosts. Genetic and biochemical studies have demonstrated that some B. cinerea small RNAs (Bc-sRNAs) can selectively silence host immunity genes by hijacking host RNAi machinery1. These Bc-sRNAs are loaded into host AGO proteins to silence host genes involved in defense. Deviated from the conventional pathogen protein effectors that suppress host immunity in plants and animals, we demonstrate that a fungal pathogen transfers "virulent" sRNA effectors into host cells to achieve infection. The implications of this finding may extend beyond grey mold disease or plant fungal diseases in general.

Funding support: NSF Career, NSF IOS, and NIH R01 to H. Jin.

1Arne Weiberg, Ming Wang, Feng-Mao Lin, Hongwei Zhao, Zhihong Zhang, Isgouhi Kaloshian, Hsien-Da Huang, Hailing Jin*: Fungal small RNAs suppress plant immunity by hijacking host RNA interference pathways. Science, 2013, 342 (6154) 118-123.

Keywords: Small RNA, AGO proteins, plant immunity, pathogen effectors, fungal pathogens
Invited Speaker #8. Plant defense: A balancing act through the immune signal salicylic acid (Submission 343)
Zheng-Qing Fu¹, Shunping Yan¹, Xinnian Dong¹
¹Duke University, United States

Plants are sessile organisms; therefore, their physiological programs are highly entrained with environmental cues. Since plant cells are polypotent, their responses to the environmental cues have to be balanced with growth and development. This balanced is maintained through sophisticated regulatory mechanisms. Under pathogen challenge, infected cells can undergo programmed cell death (PCD) to restrict pathogen growth, whereas the intact cells turn on anti-PCD genes to prevent the spread of cell death and activate systemic acquired resistance. Our recent work showed that salicylic acid (SA) produced during plant immune response plays a key role in determining cell death or survival. This is through differential SA binding to two receptor proteins, NPR3 and NPR4, and regulation of these culin3 E3 ligase interactions with the key immune regulator NPR1. During defense response, reactive oxygen intermediates (ROIs) are produced while massive transcriptional reprogramming is taking place. We found that SA can trigger DNA damage and the damage repair machinery in the absence of a geno-toxic agent. The DNA damage sensor proteins RAD17 and ATR are required for effective immune responses. Our study suggests that activation of DNA damage responses is an intrinsic component of the plant immune response.

Keywords: plant immunity, salicylic acid, programmed cell death, systemic acquired resistance, SA receptors, DNA damage response

Invited Speaker #9. Regulation of early receptor kinase-mediated immune signaling (Submission 301)
Cyril Zipfel¹
¹THE SAINSBURY LABORATORY, United Kingdom

The first layer of plant innate immunity relies on the recognition of microbes via the perception of pathogen-associated molecular patterns (PAMPs) by surface localized receptors called pattern recognition receptors (PRRs). The Arabidopsis leucine-rich repeat receptor kinases (LRR-RKs) FLS2 and EFR are the PRRs for bacterial flagellin (or flg22) and elongation factor Tu (or elf18), respectively. Within seconds of PAMP binding, FLS2 and EFR form a ligand-induced complex with the regulatory LRR-RK SERK3/BAK1 leading to phosphorylation of both proteins. FLS2 (and potentially EFR) also forms a constitutive complex with the membrane-associated cytoplasmic kinase BIK1 that get phosphorylated in a BAK1-dependent manner upon PAMP binding. BIK1 is a positive regulator of several FLS2- and EFR-mediated responses. FLS2 and EFR activation leads to several immune responses, including bursts of Ca²⁺ and reactive oxygen species (ROS), activation of mitogen-activated protein kinases (MAPKs) and calcium-dependent protein kinases (CDPKs), and transcriptional reprogramming, ultimately leading to PAMP-triggered immunity. The mechanisms controlling PRR activation at the plasma membrane and regulating intracellular signaling remain however largely unknown. Here, I will present recent work illustrating how activated PRR complexes at the plasma membrane directly engage with downstream signaling, and how these events are tightly regulated by phosphorylation.

Keywords: Innate immunity, PAMPs, PRRs, Receptor kinases, Phosphorylation

Invited Speaker #10. Type III Effectors and the Plant Immune Response (Submission 124)
Darrell Desveaux¹, Amy Lee¹, Jianfeng Zhang¹, Patrick Bastedo¹, Timothy Lo¹, Jennifer Lewis², David Guttman¹
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The plant pathogen Pseudomonas syringae causes disease on a large number of plant species using a type III secretion system to translocate effector proteins into plant cells. Many of these effectors function to suppress plant immunity. However, their recognition by plant Resistance proteins induces an effector-triggered immune response. The YopJ / HopZ family of effector proteins is a common and widely distributed effector family found in both animal and plant pathogenic bacteria. The P. syringae HopZ family includes three major allelic types (one ancestral and two brought in by horizontal gene transfer) whose diversification was driven by the host defense response. We previously demonstrated that virulence and avirulence phenotypes in Arabidopsis are allele-specific and that the ZAR1 resistance protein recognizes HopZ1a in Arabidopsis. Our recent efforts to identify the host
targets of the HopZ family are uncovering novel facets of plant immunity and pathogen virulence strategies.

Keywords: Type III secretion, Pseudomonas syringae, ZAR1, HopZ, ZED1

Invited Speaker #11. Integration of flowering time signals in Arabidopsis thaliana (Submission 319)
Markus Schmid

1MPI for Developmental Biology, Germany
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. Precocious flowering often results in reduced yield, both in biomass and fruits. In contrast a delay in flowering can result in an increase of biomass. Because of its importance, flowering is under the control of a complex genetic circuitry that integrates endogenous signals such as hormonal and carbohydrate status, and environmental signals such as temperature and light. Genetic analyses initially suggested the existence of distinct genetically defined pathways that perceive and regulate flowering in response to a specific input. For example, the FLOWERING LOCUS T (FT) gene, a key regulator of flowering time, is induced in the leaf vasculature in response to permissive day length and acts as a long distance signal (florigen) that transmits the information to initiate flowering from leaves to the shoot apical meristem. Over the last several years, however, it has become apparent that genes such as FT are not regulated by single inputs, but rather integrate multiple, often contradictory signals to control the induction of flowering. Consequently, these genes are nowadays often referred to as "integrator genes". Here I will discuss the role of the disaccharide trehalose-6-phosphate and changes in ambient temperature in modulating the expression of integrator genes such as FT to ensure that flowering commences under optimal conditions.

Keywords: Arabidopsis thaliana, flowering time, signal integration

Invited Speaker #12. Entrainment of Arabidopsis circadian oscillators by sugars (Submission 297)
Alex Webb

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Arabidopsis circadian clocks confer advantage by generating rhythmic activity of metabolism, physiology, signalling and developmental processes. Arabidopsis circadian oscillators are thought to be formed of transcription-translation feedback loops and associated cell physiological processes. To ensure that the timing of internal events is correctly matched to the local environment the phase of circadian rhythms are set by light and temperature signals at dawn in a process called entrainment. For examples rhythms of photosynthesis are phased such that the peak activity occurs during the day. We have recently shown that rhythmic endogenous sugar signals due to photosynthetic activity entrain circadian rhythms in Arabidopsis by regulating circadian clock gene expression. We have proposed that phase adjustment in response to sugar signals defines a 'metabolic dawn' which occurs approximately 4h after lights on. Endogenous oscillations of sugars provide metabolic feedback to the circadian oscillator through the morning-expressed PSEUDO RESPONSE REGULATOR 7 (PRR7) and CIRCADIAN CLOCK ASSOCIATED 1 (CCA1). prr7 and cca1 mutants are insensitive to the effects of sugar. We are investigating the molecular mechanisms that generate metabolic dawn and why plant circadian oscillators entrain to sugar signals.

Keywords: circadian, photosynthesis, entrainment, Arabidopsis, sucrose, sugars

Invited Speaker #13. Xylem Cell Development and Differentiation in Plant Roots (Submission 497)
Siobhan Brady

1UC Davis, United States
Roots are of fundamental importance for both plant and human growth and health. Distinct cell type-specific programs within the root perform a variety of functions including defense, nutrient transport, mechanical support and growth. Root spatiotemporal transcriptome, proteome and metabolome profiling in Arabidopsis thaliana roots have given great insight into the diversity of cell type-specific programs. I will highlight how we can use these data and other genome-scale and systems biology approaches to map gene regulatory networks that regulate xylem cell development in Arabidopsis thaliana, tomato and sorghum.
Invited Speaker #14. -Omics responses to abiotic stress in Arabidopsis (Submission 352)
Sarah Assmann1, Biswa Acharya1, Karim Osman1, Yiliang Ding1, Yin Tang1, Chun Kit Kwok1, Reka Albert1, Philip Bevilaqua1
1Penn State University, United States
As sessile organisms that cannot escape unfavorable conditions, plants have evolved numerous mechanisms that confer tolerance to abiotic stresses, including drought stress. In vascular plants, stomatal closure in response to the stress hormone abscisic acid (ABA) reduces transpirational water loss and is one of the key components of drought tolerance. ABA activates an intricate cellular signaling network which includes changes in the guard cell transcriptome, proteome, and metabolome and ultimately results in cell volume loss and stomatal closure. We have characterized these guard cell -omics responses to ABA and are working on updating our published dynamic model (Li et al. PLoS Biol., 2006) of guard cell ABA signaling. In addition to the transcriptome, proteome, and metabolome, abiotic stresses can also change the physical structure of RNA. We have recently developed a method, Structure-Seq, which allows genome-wide probing of RNA secondary structure in vivo at single nucleotide resolution. Results from our Arabidopsis in vivo RNA structurome (Ding et al., Nature, 2014) indicate that stress-related mRNAs have structural features that may facilitate their conformational change in responses to stress while housekeeping-related mRNAs tend to have one thermodynamically favored structure. These results raise the hypothesis that RNA structure and its modulation may be an important and heretofore little recognized component of abiotic stress response in plants.

Keywords: root development, networks, systems biology

Invited Speaker #15. Smarty Plants: Using Mechanosensitive Ion Channels to Sense and Respond to Mechanical Force (Submission 276)
Elizabeth Haswell1
1Washington University in Saint Louis, United States
A long-standing problem is how biological systems sense and perceive mechanical signals such as osmotic pressure, gravity, and touch. One well-established molecular mechanism for force sensing at the cellular level is the activation of mechanosensitive (MS) ion channels. The Mechanosensitive channel of Small conductance (MscS) from E. coli functions as a hypo-osmotic safety valve, opening in response to increased membrane tension and preventing cellular rupture. Genes predicted to encode MscS homologs are found in all three kingdoms of life. We have been characterizing the structure, function, and regulation of ten MscS-Like (MSL) proteins in Arabidopsis thaliana. Based on their modest homology to MscS and high topological diversity, we have proposed that MSLs might (1) sense and respond to sources of membrane tension other than environmental hypo-osmotic shock; (2) be regulated by mechanisms in addition to membrane tension; and (3) signal in ways that are separable from ion flux. Evidence in support of all three of these hypotheses will be presented.

Keywords: signal transduction, ion channel, force perception

Invited Speaker #16. Microtubule-mediated control of auxin polar transport and brassinoteroid signalling (Submission 379)
Yuan Ruan1, Chris Ambrose1, Geoffrey Wasteneys1
1University of British Columbia, Canada
CLASP (Cytoplasmic Linker Protein Associated Protein) is a highly conserved microtubule-associated protein with well-defined functions in numerous model systems. These include microtubule rescue, kinetochore function and microtubule plus end capture at the cell cortex during cell migration. In plants, CLASP has both conserved and specialized roles. It is involved in maintaining microtubule contact with the plasma membrane and, through its association with cell edges, it coordinates microtubule organization on difference cell faces. The latter function appears to be critical for maintaining an adequate population of cells in the meristematic regions of plants, consistent with mutants lacking CLASP gene expression having fewer and smaller cells. Recently it has been shown that CLASP interacts with the retromer complex component sorting nexin 1 (SNX1). This interaction serves
to tether SNX1 endosomes to cortical microtubules, which serves to maintain high levels of the auxin efflux carrier PIN2. Thus, auxin polar transport is aberrant in clasp-1 mutant, which may help to explain the dwarf phenotype. We are currently investigating the possibility that CLASP is both regulated by the brassinosteroid signalling pathway and that it, in turn, can modulate brassinosteroid signalling. Consistent with the CLASP promoter containing a sequence that is a known target of the brassinosteroid-stimulated transcriptional repressor BZR1, CLASP expression in roots is suppressed by brassinolide treatment. In addition, the activity of the brassinosteroid receptor BR1, which has been reported to be a cargo of the SNX1 retromer complex, appears to be altered in the clasp-1 mutant. These findings suggest that CLASP has evolved to become integrated in the cross-talk between auxin and brassinosteroid signalling.

Keywords: hormone, ethylene signaling, metal ion transport, phosphorylation

Invited Speaker #17. Regulation of the central regulator EIN2 in ethylene hormone signaling in Arabidopsis
(Submission 355)
Jennifer Shemansky1, David Lin1, Chuanli Ju1, Sebastien Thomine2, Caren Chang1
1University of Maryland, United States, 2CNRS, France
The plant hormone ethylene has profound effects on plant growth and development, including fruit ripening, abscission, senescence and responses to biotic and abiotic stresses. In the ethylene signaling pathway, a central component, ETHYLENE INSENSITIVE2 (EIN2), localizes to the ER membrane along with the ethylene receptors, but the molecular mechanisms of EIN2 signaling have long remained a mystery. EIN2 consists of two domains that are largely uncharacterized: an ER membrane-localized N-terminal domain, which has sequence similarity to Nram metal ion transporters, and a novel hydrophilic C-terminal domain that is required for downstream signaling. Our previous studies showed that in the absence of ethylene, the CTR1 protein kinase phosphorylates the EIN2 C-terminal domain to prevent signaling, whereas in the presence of ethylene, the lack of phosphorylation by CTR1 results in cleavage of the EIN2 C-terminal domain from the membrane-bound N-terminal domain followed by translocation into the nucleus where it activates downstream signaling. These findings have filled the gap between the ER membrane and the nucleus in ethylene signaling, while resolving the long-standing controversy over the identity of the CTR1 kinase substrate. More recently, we have obtained evidence that the EIN2 N-terminal domain may transport a metal ion, and such transport may play a role in positively regulating the C-terminal signaling domain. These findings, for the first time, link metal ion transport with ethylene signaling by EIN2.

Keywords: hormone, ethylene signaling, metal ion transport, phosphorylation

Invited Speaker #18. Friends on surface, foes underground - the complicated relationship between brassinosteroid and auxin
(Submission 493)
Eunkyoo Oh1, Juthamas Chaawanon1, Jia-Ying Zhu1, Zhiyong Wang2
1Carnegie Institution for Science, DPB, United States, 2Carnegie Institution for Science, United States
In plants, one hormone often induces different cellular responses in different organs and tissues, whereas one cellular response is often controlled by combinations of hormones. For example, hypocotyl elongation is synergistically promoted by three growth hormones, brassinosteroid (BR), auxin, and gibberellin (GA), but is inhibited by light. We recently found that such co-regulation of hypocotyl elongation is mediated by a circuit of interacting transcription factors controlled by these hormonal and light signals. The BR-activated BZR1 transcription factor, the auxin-activated ARF6, and the light-sensitive PIF4 interact cooperatively at hundreds of shared gene promoters to activate gene expression interdependently. However, their activities depend on GA because their DNA binding activities are all inhibited by the GA-sensitive DELLA proteins. The requirement of PIFs for BZR1 and ARF6 function explains why the hormone sensitivities are modulated by factors that alter PIF levels, such as light, the circadian clock, temperature, and the PIF-interacting HLH proteins. The interaction between BZR1 and ARF6 explains the long observed BR-auxin synergism in shoot development. Surprisingly, but not unexpectedly, we found that BR and auxin act antagonistically in root growth, with opposite spatial gradient and effects on gene expression and cell elongation. I will discuss why and how BR-auxin relationship is turned upside down from shoots to roots.
Invited Speaker #19. Polymerase IV occupancy at RNA-directed DNA methylation sites requires SHH1 (Submission 305)
Julie A Law¹, Jiamu Du¹, Christopher J Hale³, Suhua Feng³, Ana Marie S Palanca¹, Krzysztof Krajewski², Brian D Strahl², Dinshaw J Patel², Steven E Jacobsen³
¹Salk institute, United States, ²Memorial Sloan-Kettering Cancer Center, United States, ³University of California at Los Angeles, United States
In eukaryotic organisms DNA is organized into chromatin, a dynamic structure consisting of mainly DNA and specialized packaging proteins called histones. Additional information can be layered on top of chromatin through the covalent attachment of chemical groups to DNA and/or histone proteins, and these modifications influence the expression of the underlying genes and play critical roles in normal growth and development. One such modification is DNA methylation, which modifies cytosine bases and is associated with transcriptional gene silencing. In Arabidopsis, DNA methylation is established by DOMAINS REARRANGED METHYLENSFERASE 2 (DRM2) and is targeted by 24 nt small interfering RNAs (siRNAs) through a pathway termed RNA-directed DNA methylation (RdDM). A key step in the RdDM pathway is the biogenesis of the DNA methylation-targeting siRNAs, which is initiated by the activity of the RNA polymerase IV (Pol IV) complex. To understand the mechanisms controlling Pol-IV targeting, we investigated the role of SAWADEE HOMEOIDOMAIN HOMOLOG 1 (SHH1), a Pol-IV associated factor previously shown to affect DNA methylation and siRNA levels. Using a combination of genetic, genomic, and biophysical techniques we were able to demonstrate that SHH1 functions to enable siRNA production at specific genomic loci by facilitating Pol IV recruitment and that this recruitment requires the SHH1 SAWADEE domain, a tandem Tudor-like domain that functions as a dual methyl-lysing reader. Consistent with a role in gene silencing, the SAWADEE domain recognizes histone 3 tails that are unmethylated at lysine 4 (H3K4) and methylated at lysine 9 (H3K9me). Together, these findings connect DNA methylation and H3K9 methylation within the context of the RdDM pathway and provide the first mechanistic insight into a key initiating step in the RdDM pathway. * First three authors contributed equally.

Keywords: DNA methylation, siRNA, Tudor domain, gene silencing, chromatin

Invited Speaker #20. Epigenetic control of meiotic crossover hotspots in Arabidopsis (Submission 288)
Ian Henderson¹
¹University of Cambridge, United Kingdom
Meiotic crossover creates genetic diversity and underpins genetic analysis and crop breeding. It has long been appreciated that crossover frequency is non-random in genomes with the presence of hotspots and coldspots. Using population genetic approaches we have generated maps of historical crossover frequency in the Arabidopsis genome. This revealed that crossovers are concentrated at gene transcriptional start and termination sites, consistent with correlations between gene density and recombination observed in other plants. Hotspot gene promoters associate with epigenetic marks enriched on transcriptionally active genes and using experimental genetics we have demonstrated that H2A.Z is required for normal crossover frequency. More recently we have used RNA-directed DNA methylation to silence crossover hotspots, further demonstrating the importance of chromatin for recombination. I will present new data on our use of genome-wide approaches to map meiotic recombination proteins, including the SPO11 endonuclease, and what this is revealing about control of crossover locations and frequency. A greater understanding of how DNA sequence and epigenetic information modulate meiotic recombination will find useful applications during crop breeding.

Keywords: Meiosis, Genetics, Epigenetics, Recombination

Invited Speaker #21. Agrichemical Control of Drought Tolerance using Engineered ABA Receptors (Submission 489)
Sang-Youl Park¹, Francis Peterson², Assaf Mosquna³, Jin Yao¹, Brian Volkman², Sean Cutler¹
¹University of California, United States, ²Medical College of Wisconsin, United States, ³Hebrew University of Jerusalem, Israel

Rising temperatures and lessening water supplies are threatening agricultural productivity and have motivated efforts to improve plant water use and drought tolerance. During water deficit, plants produce elevated levels of abscisic acid (ABA), which improves water consumption and stress tolerance by controlling guard cell aperture and other protective responses. One attractive strategy for controlling water use is to develop compounds that activate ABA receptors, but agonists approved for use have yet to be developed. In principle, ABA receptors engineered to be responsive to an existing agrichemical would enable chemical control by a molecule in current use. Here, we describe an engineered variant of the ABA receptor PYR1 that possesses nanomolar sensitivity to the agrichemical mandipropamid and demonstrate its efficacy for controlling ABA responses and drought tolerance in transgenic plants. Furthermore, crystallographic studies provide a mechanistic basis for its activity and demonstrate the relative ease with which the PYR1 ligand-binding pocket can be altered to accommodate new ligands. Thus, we have successfully repurposed an existing agrichemical for a new use through receptor engineering. We anticipate that this strategy can be applied to other plant receptors and opens new doors for crop improvement.

Keywords: Abscisic acid, Drought tolerance, Abiotic stress, Synthetic biology, Chemical genomics

Invited Speaker #22. Regulatory Networks Controlling Hormone-Mediated Growth (Submission 490)
Joseph Ecker¹
¹HHMI, The Salk Institute, United States

Plant growth is a dynamic process that requires active orchestration of spatial and temporal patterns of cellular behavior and coordination of gene expression programs. In addition to external environmental cues such as changing light and temperature, internal cues such as small molecule growth regulators (also called plant hormones) play pivotal roles in the growth and developmental processes. A major challenge in understanding the roles of plant hormones in such processes is the lack of knowledge concerning how cells interpret complex spatio-temporal signals to regulate gene expression patterns and ultimately cell behavior. Genetic analyses based on terminal phenotypes have allowed identification of the major components of most hormone signaling pathways in Arabidopsis. Moreover, subsequent biochemical analyses have helped to define the functions of individual genes in each of the hormone signaling pathways. However, these classical approaches have largely been unsuccessful in identifying genes downstream of each hormone-signaling pathway that ultimately regulate and coordinate growth networks. This may be because of the robust nature of growth pathways where altered activity of one component in one signaling pathway affects the entire network and therefore, the ultimate mutant phenotype represents a cumulative effect of a series of events that affects the expression of many genes as well as functional overlap among genes in families.

Our recent research efforts attempt to uncover transcriptional factor (TF) networks for each of the major plant hormones including abscisic acid (ABA), auxin (IAA), brassinosteroids (BR), cytokinin (CK), ethylene (ET), gibberellins (GA), jasmonate (JA), salicylic acid (SA), and strygolactone (SL). We are applying genomic, epigenomic and proteomic tools to identify and annotate gene regulatory elements and identify new components in each hormone-signaling pathway. The immediate outcome of this research will result in the creation of dynamic plant hormone interaction network maps in actively growing seedlings for each of the major plant hormones. The long-term goal of our research is to utilize the dynamic transcriptional network maps to develop predictive models of hormone-mediated seedling growth.

Keywords: Transcription factors, ChIP-seq, RNA-seq

Invited Speaker #23. Auxin Perception and Response in Arabidopsis and Moss (Submission 473)
Mark Estelle¹
¹UCSD, United States

Auxin regulates a bewildering array of processes during plant growth and development. This complexity contrasts with the apparent simplicity of the auxin-signaling pathway. Auxin regulates transcription through the TIR1/AFB-Aux/IAA-ARF pathway. The hormone directly binds to the F-box protein of the SCFTIR1/AFB ubiquitin protein ligase E3 and promotes an interaction with the Aux/IAA transcriptional repressors, thus stimulating their degradation. Loss of the Aux/IAAs permits ARF-dependent transcription. In the case of the TIR1/AFB proteins,
recent results indicate that different members of the family have distinct activities both with respect to auxin binding and Aux/IAA interaction. We are currently exploring the possibility that these differences contribute to the complexity of auxin response. In addition we have used the yeast 2-hybrid system to isolate tir1 mutants that illustrate novel aspects of TIR1 function and regulation. Finally, we are investigating the downstream transcriptional networks that mediate auxin-dependent growth responses.

Keywords: auxin, ubiquitin, physcomitrella patens

Invited Speaker #24. Using Insights from Basic Research and Arabidopsis to Develop Improved Wheat Varieties (Submission 327)
Peggy G. Lemaux

Thioredoxin (Trx), a small molecular weight protein, catalyzes reactions via a dithiol-disulfide exchange mechanism involving a site with two redox-active cysteine residues separated by two amino acids. This arrangement leads to easy access of the Trx redox center to the disulfide (S-S) bridges of specific target proteins, thus facilitating changes in their structure and activity. Trxs are present in all living organisms; however, Trxs in plants belong to a large, complex family, present in different cell compartments. In Arabidopsis, there are 19 members - two in mitochondria (Trx o), nine in chloroplasts (Trx m, f, x, y) and eight in the cytosol (Trx h). Initial work in plants centered on the role of Trx in chloroplasts where it was demonstrated that reduction of Trx is linked to photosynthesis. Further work, primarily in cereals, established the role of cytosolic Trx h as a central regulatory protein in seeds, where it reduces disulfide groups in diverse, yet specific, seed storage proteins, enzymes and enzyme inhibitors. These early in vitro protein studies were complemented with studies in which Trx h5 was overexpressed in cereal grain endosperm. In barley grain, embryo germination was accelerated and starch-degrading enzymes were expressed early or were enhanced, traits important to the malting and brewing industry. Later transgenic approaches in wheat altered Trx h expression in different ways. In one study, Trx h5 overexpression, driven by an endosperm-specific promoter and its protein body targeting sequence, led to increased solubility of disulfide proteins and decreased allergenicity. In a second study in wheat, an antisense Trx h9 construct, driven by another endosperm-specific promoter, resulted in underexpression of that gene in the cytosol. In this case engineered grain germinated slower with a dramatic delay in preharvest sprouting. Importantly, baking quality was identical to that of nonengineered grain. Unexpectedly, wheat spikes from this engineered variety, even in the absence of humidity, had increased grain yield. Clues to the mechanisms by which changes in the endosperm resulted in effects in a different seed compartment, the embryo, in both barley and wheat awaited studies on Trx h9 in Arabidopsis. Using an endoderm-specific promoter and GFP-fusions, Trx h9, unlike other Trxs, was shown to be capable of moving from cell-to-cell, thus providing a mechanism for intercompartmental communication of redox state.

Keywords: Thioredoxin, Redox State, Improved wheat varieties

Invited Speaker #25. Spinning straw into gold - translating fundamental lignin research into application (Submission 342)
Claire Halpin

Plant biology is once again in the ascendancy in the effort to discover solutions to impending global problems of food security, sustainable energy provision, and climate change. The advent of the genomics era and sequencing of the Arabidopsis genome, along with the availability of off-the-shelf Arabidopsis mutants, have revolutionized plant research and provided unprecedented tools for determining the functions of individual genes and the pathways and mechanisms by which they interact. These fundamental insights increasingly underpin translational research in a variety of crops. My research focuses on lignin biosynthesis and how it can be manipulated to improve plant biomass for a variety of industrial applications, particularly second generation biofuel production. Detailed knowledge of the entire complement of genes constituting the lignin biosynthesis pathway in Arabidopsis has served as a robust starting point to allow us to explore the pathway in barley, the world's fourth most important cereal crop. By identifying barley genes that are orthologous to lignin biosynthesis genes known in
Arabidopsis and then modifying their expression using silencing technologies, varieties with novel biomass composition have been generated and tested for utility in different processes. A second approach has been to explore and exploit natural variation in various elite or wild barley populations to identify quantitative trait loci that underlie lignin content or composition, and other traits influenced by lignin such as straw strength, straw digestibility and disease resistance. Several aspects of this work have brought us back to fundamental gene discovery in Arabidopsis. My talk will highlight both the opportunities and limitations presented by Arabidopsis research for facilitating translational work to improve barley straw for second generation biofuel and chemical production.

Keywords: Lignin, 2nd generation biofuels, Barley

Invited Speaker #26. Phased, secondary siRNAs in plants (Submission 316)
Blake Meyers1
1University of Delaware, United States

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). Secondary siRNAs, produced in a phased pattern and thus often called phasiRNAs, apparently play important roles in post-transcriptional control of many non-coding and protein coding RNAs. This is true in Arabidopsis, but even more pronounced in non-Arabidopsis plant species. For example, in soybean, we have identified more than 500 phased ("PHAS") loci, which 483 loci overlapped annotated protein-coding genes. The primary class of PHAS in most dicot genomes corresponds to NB-LRR genes. From detailed analysis in soybean, we have found that phasiRNAs, and known and novel miRNAs are often expressed preferentially to particular tissues and treatments. Separately, our analysis of maize and rice anthers has identified two novel classes of highly abundant small RNAs. We used developmentally staged anthers to show that one class of phased, secondary siRNAs (‘phasiRNAs’) emerges abruptly in pre-meiotic stages during cell fate specification. A second class, generated by a specialized, monocot-specific DICER, emerges coordinately with germinal cell maturation and meiosis, persisting into mature pollen. Male-sterile mutants distinguish these classes and demonstrate that there are distinct regulatory features of these small RNAs, both spatially and temporally. Thus the grasses contain analogs of animal piRNAs: one class of small RNAs participating in pre-meiotic development and a second related to meiosis. From dicots to monocots, we have found many unusual roles for phased, secondary siRNAs.

Keywords: microRNAs, small RNAs, Translational biology

Invited Speaker #27. Stomatal Patterning: Communication, Fate, and Decision making (Submission 310)
Keiko U. Torii1
1HHMI and University of Washington, United States

Multicellular organisms produce complex tissues with specialized cell types for optimal functionality. Our group is interested in understanding the molecular mechanism of how a cell constituting a multicellular organism communicates with each other to execute decision-making processes. Specifically we are focusing on stomatal patterning on developing epidermis as a model for two-dimensional spatial patterning for its simplicity, accessibility, and availability of molecular-genetic and imaging tools. Stomatal patterning occurs according to positional cues and requires critical cellular decision-making steps of whether or not to become stomata. During development of photosynthetic organs, a selected population of undifferentiated protodermal cells undergoes asymmetric cell divisions that initiate the stomatal cell lineage. A stomatal precursor cell reiterates asymmetric cell division and eventually differentiates into guard cells. Recent progress by our group and others has led to the discovery of key molecules and pathways controlling stomatal patterning and differentiation: (1) Sequential and combinatorial actions of five bHLH transcription factors specifying stomatal precursor cell state transitions; (2) A ligand-receptor system enforcing frequency and orientation of asymmetric cell division; (3) Intrinsic polarity and cellular constituents that are required for creating and maintaining asymmetry. The next challenge is to understand how these regulatory components: ligands, receptors, transcription factors and polarity components are put together in the context of two-dimensional tissue patterning in the plant epidermis. Combining high-
resolution live imaging approaches, biochemistry, mathematical modeling, and large-scale genomics and epigenomics, we seek to unravel multi-scale regulatory mechanisms coordinating stomatal patterning in real time and space.

Keywords: Peptide signaling, Receptor Kinases, Stomatal Patterning, Cell fate specification, Transcriptional control

Invited Speaker #28. Overlap between developmental patterning pathways and abiotic stress response pathways. (Submission 499)
M. Kathryn Barton
1Carnegie Institution for Science, United States

It is well accepted that abiotic stresses, such as lack of water, can modify plant growth and development. In practice, however, it has been difficult to understand which developmental regulatory channels these stresses act through. In the course of defining the ad/abaxial regulatory network we have identified points where this developmental network regulates abscisic acid (ABA) signaling genes and vice versa. While the ad/abaxial transcriptional regulators are best known for their transformative roles in leaf polarity, they also play major roles in regulating growth. The HDZIPIII genes promote new shoot apical meristem formation and organ formation while the KANADI genes inhibit shoot apical meristem formation and organ formation. We have found that ABA mediated inhibition of vegetative shoot apical meristem activity in growing plants requires KANADI action. Furthermore, HDZIPIII and KANADI genes act downstream of ABA to promote and inhibit germination, respectively. We have exploited the discovery of the interaction between the two networks to identify new players in the vegetative ABA signaling pathway and to generate new genetic materials that mediate drought resistance. (This work was funded by the National Science Foundation and by the Carnegie Institution for Science.)

Keywords: leaf polarity, shoot apical meristem, leaf development, ABA, abscisic acid, germination, REVOLUTA, KANADI, HDZIPIII, abiotic stress

Invited Speaker #29. Dynamics of stem cell signalling pathways in meristems (Submission 322)
Rudiger Simon
1CEPLAS Heinrich-Heine University, Germany

Organogenesis in plants is based on precise cell fate determination of undifferentiated cells, which are derived from stem cells that reside in meristems. Cell fate determination depends on the position of a cell within the plant body. A number of mechanisms provide positional information to cells or cell populations: (1) plant hormones like auxin establish gradients or local concentration maxima; (2) secreted ligands and membrane associated receptors enable cell-cell signalling between adjacent cell layers; (3) transcription factors can act non-cell autonomously to integrate cellular responses, and (4) long range communication can take place by transport of signals between cells through plasmodesmata.

Our current understanding is that these mechanisms act in a concerted manner to govern plant development. I will report on our most recent results on receptor kinase signalling pathways that control the growth and maintenance of meristems. Using a toolbox of fluorescence imaging techniques in planta, we studied the dynamics of receptor assembly into larger complexes at distinct regions of the plasmamembranes upon the perception of their specific ligands. Furthermore, we found that alterations of receptor expression levels can have profound developmental consequences. Finally, I will link aspects of peptide-receptor and hormonal signalling pathways.

Keywords: receptor, meristem, signalling

Invited Speaker #30. Gibberellin acts positively then negatively to control onset of flower formation in Arabidopsis (Submission 302)
Nobutoshi Yamaguchi1, Cara Winter1, Miin-Feng Wu1, Yuri Kanno2, Aayako Yamaguchi1, Mitsunori Seo2, Doris Wagner1
1University of Pennsylvania, United States, 2Riken Research Institute, Japan

The switch to reproductive development is biphasic in many plants, a feature important for optimal pollination and
yield. We show that dual opposite roles of the phytohormone gibberellin underpin this phenomenon in Arabidopsis. While gibberellin promotes termination of vegetative development, it is inhibitory for floral fate. To overcome this effect, the transcription factor LEAFY induces expression of a gibberellin catabolism gene, consequently increased LEAFY activity causes reduced gibberellin levels. This allows accumulation of gibberellin-sensitive DELLA proteins. The DELLA proteins are recruited by SQUAMOSA PROMOTER BINDING PROTEIN LIKE transcription factors to regulatory regions of the floral commitment gene APETALA1 and promote APETALA1 upregulation and floral fate synergistically with LEAFY. The two opposing functions of gibberellin may facilitate evolutionary and environmental modulation of plant inflorescence architecture.

Keywords: Switch to flower formation, Gibberellin, LEAFY, DELLA, SPL proteins, Inflorescence architecture

Invited Speaker #31. Gamete activation during double fertilization (Submission 494)
Frank Vogler¹, Maria Englhart¹, Philipp Denninger², Andrea Bleckmann¹, Philipp Cyprys¹, Guido Grossmann², Thomas Dresselhaus¹, Stefanie Sprunck²
¹University of Regensburg, Germany, ²University of Regensburg and Universitat Heidelberg, Germany

Flowering plants have evolved the unique mechanism of double fertilization in which two female reproductive cells are fertilized by two male gametes. The two gamete-fusion events occur deep in the maternal tissues of the ovary and ovule. The ovule protectively surrounds the female haploid generation, termed embryo sac, which in Arabidopsis consists of two female reproductive cells (egg cell and central cell), and two types of accessory cells (synergid and antipodal cells). Typically, one pollen tube enters the ovule, bursts upon successful interaction with one of the synergid cells, and delivers the two non-motile sperm cells to the vicinity of the two female gametes. Approximately five minutes later one sperm cell starts to fuse with the egg cell, while the second sperm cell fertilizes the central cell.

Across phyla, fertilization occurs through a series of coordinated steps, mediated by molecules that act on the gametes surfaces. Gamete interactions include cell recognition, cell adhesion and then controlled fusion of the gamete plasma membranes. Likewise, reciprocal cell-induced sperm and egg activation are central events during fertilization, being important for the fusogenicity of the sperm plasma membrane and the eggs blocks to polyspermy. Finally, the role of fertilization-induced release of calcium ions in egg activation has become evident in several animal species.

But what happens when the two Arabidopsis sperm cells meet the two female gametes? We still know very little about the molecular events that take place in the few minutes between sperm cell delivery and gamete fusion. We will report here on our transcriptomics approaches in isolated female gametes of wheat and Arabidopsis, which have led to the identification of small cysteine-rich EC1 (EGG CELL 1) proteins secreted by the egg upon sperm cell delivery to achieve rapid sperm activation and gamete fusion. By monitoring the cytosolic Ca2+ dynamics in the female gametes of Arabidopsis using live-cell imaging and the troponin C-based calcium sensor CerTN-L15 we furthermore provide strong evidence for a role of Ca2+ signaling during double fertilization as each female gamete displays a characteristic calcium signature differing by timing and behaviour from cytoplasmic Ca2+ waves reported in animals.

Keywords: egg cell, sperm cell, gamete fusion, triggered exocytosis, calcium

Invited Speaker #32. Dissecting Quantitative Regulation of Root Growth Using Large-Scale Phenotyping and Systems Genetics (Submission 333)
Wolfgang Busch¹
¹Gregor Mendel Institute of Molecular Plant Biology, Austria

One of the ultimate challenges of biology is to understand how genotypes translate into phenotypes. Recent technological leaps in DNA sequencing have brought tremendous progress in this area. However, compared to the progress on acquiring genome sequences, our comprehension of genotype to phenotype relations is growing very slowly, especially in complex multicellular organisms such as plants, in which specific biological processes often occur only temporarily and are restricted to specific organs, tissues or even individual cells. One bottleneck is the ability to accurately phenotype a large number of individuals and genotypes in a temporally and spatially resolved manner. Another major intricacy is to identify and comprehend dynamic networks of interacting genes.
As a model to address these challenges, we use the root of Arabidopsis thaliana and try to dissect how root growth is tuned by the activities of genes and their networks. For this we have developed several high-throughput methods to accurately quantify multiple traits describing root growth and development over periods of time and at different scales. With these pipelines, we assess the trait variation that is present in natural and artificial populations of Arabidopsis. Using genome-wide association mapping combined with the analysis of expression and other large-scale -omics data, we determine genes, their alleles and the networks that act to quantitatively regulate root developmental and growth processes. With these methods we have recently identified multiple novel regulators and networks that tune root growth.

Keywords: root growth, development, quantitative genetics

Invited Speaker #33. Quantitative tools to understand the mechanics of morphogenesis (Submission 280)
Olivier Hamant

ENS Lyon, France

In developmental biology, the accumulation of qualitative phenotypic descriptions has fueled the need for testable parsimonious hypotheses, giving a fresh impetus to quantitative strategies. Beyond the quantification of growth, molecular marker expression and hormone levels in tissues, quantifying mechanical forces and their effect is particularly tricky as forces are not visible in essence. Here I will focus on how to approach the role of mechanical signals in plant development using 1/ microtubule orientation and anisotropy indexes as stress sensors, 2/ micro and nanoindentation outputs as quantitative measurement of cell and cell wall stiffness, and 3/ modeling approaches to predict stress patterns, using Arabidopsis pavement cells and shoot meristems as model systems.

Keywords: mechanical stress, development, meristem, pavement cell, AFM, microtubule, modeling, biophysics

Invited Speaker #34. Arabidopsis as a model to identify genes to overcome biomass recalcitrance for biofuels (Submission 344)
Wout Boerjan

VIB, Belgium

Lignin is an aromatic polymer that is abundantly present in secondarily-thickened plant cell walls. It plays a negative role in a number of agro-industrial processes, such as chemical pulping and it is also one of the most important limiting factors in the conversion of plant cell walls to fermentable sugars in the process to liquid biofuels. Hence, significant research efforts have been devoted to understand the lignin biosynthesis pathway. These studies have demonstrated that altering lignin amount or composition can improve the processing of plant biomass. We have shown that Arabidopsis is a good model for biofuel research. Senesced inflorescence stems can be enzymatically processed (saccharified) into glucose, and mutants that deposit less lignin are more easily saccharified. We then have used a systems biology approach in which 20 mutants in the lignin biosynthesis pathway were compared side by side by transcriptomics and metabolomics. These data have revealed i) how the plant responds, as a biological system, to pathway perturbations and ii) how the lignin biosynthesis pathway is embedded into the wider plant metabolism. In addition, the data set revealed a number of unknown genes that were closely co-expressed with known lignin biosynthesis genes. One of these, CSE, turned out the encode an esterase involved in the lignin biosynthesis pathway itself. Metabolic profiling of the cse mutants revealed a strong accumulation of caffeoyl shikimate, a known intermediate of the lignin biosynthesis pathway. Enzyme assays with heterologously expressed CSE demonstrated that it was the natural substrate of the enzyme, hence, CSE turned out to be an enzyme central to the lignin biosynthesis pathway. cse mutants deposited 36% less lignin. Associated with the changes in lignin, the conversion of cellulose to glucose in cse mutants increased up to fourfold as compared to the wild type upon saccharification, opening perspectives to engineer the expression of orthologs in bioenergy crops. Together, these data necessitated the revision of the lignin biosynthetic pathway. Vanholme R., Cesarino I., Rataj K., Xiao Y., Sundin L., Goeminne G., Kim H., Cross J., Morreel K., Araujo P., Welsh L., Hausstraete J., McClellan C., Vanholme B., Ralph J., Simpson G.G., Halpin C.* and Boerjan W.* (2013). Caffeoyl shikimate esterase (CSE) is an enzyme in the lignin biosynthetic pathway in Arabidopsis. Science 341, 1103-1106.
Keywords: lignin, cell wall, biomass, metabolomics, phenolics, saccharification, bioethanol, biosynthetic pathway, systems biology

**Invited Speaker #35. Meristem activity, Inflorescence form and yield of rice** (Submission 453)
Junko Kyozuka¹, Hiroki Tokunaga¹, Akiko Yoshida¹
¹Graduate School of Agriculture and Life Sciences, University of Tokyo, Japan

The number of grains produced per inflorescence is a critical determinant of yield in gramineous crops including rice, maize and wheat. Flowers form in a spikelet, or small branch in the Gramineous species. The spikelet is unique to the grass species and considered as the basic unit defining the inflorescence structure of these species. Recent progress in molecular genetic studies in model grass species, such as rice and maize, has enabled the isolation of key regulators controlling grass inflorescence form. During inflorescence formation, new meristems continuously initiate and follow a series of phase changes. Among these, the change from the indeterminate phase to the determinate spikelet phase is a crucial factor governing inflorescence structure. During rice inflorescence development, newly formed meristems acquire a branch meristem identity to generate further meristems or terminate as a spikelet. Thus, the inflorescence form of rice is determined by the reiterative pattern of decisions made at meristems. In the dominant gain-of-function mutant tawawa1-D (taw1-D), a delay in spikelet specification causes prolonged branch formation, resulting in increased grain production. We show that the taw1-D2 allele mediates more than a 10% rise in yield in a commercial rice cultivar. In contrast, reduction of TAW1 activity accelerates spikelet formation resulting in a decrease in grain number. We thus propose TAW1 as a novel molecular link between grain number and meristem phase change in rice. TAW1 encodes a nuclear protein of an unknown function. The contribution of TAW1 for crop yield and its molecular function will be discussed.

Keywords: TAW1, meristem phase change, improving crop yield

**Invited Speaker #36. Gene Regulatory Networks Mediating Arabidopsis Responses to Environmental Stress** (Submission 484)
Katherine Denby¹
¹University of Warwick, United Kingdom

Plant responses to abiotic and biotic stress involve large-scale transcriptional reprogramming. We are elucidating transcriptional networks underlying these environmental responses using a combination of experimental and computational/mathematical tools. We generated high-resolution time series expression data from Arabidopsis leaves following pathogen infection (bacterial and fungal), drought, and high light. This data has enabled us to resolve the chronology of these environmental responses and identify transient responses. We have generated transcriptional network models predicting regulatory relationships between differentially expressed transcription factors and identified key regulators of these responses from our networks. Network comparison has highlighted interactions common to multiple responses and those that appear to be more response-specific. The network models are being extended by identifying groups of genes co-regulated across multiple stress responses and validated by experimentally testing regulatory predictions from model simulations. I will highlight the biological insight we have gained from our time series expression profiling and network modelling approach and tools we have developed to gain this insight.

Keywords: Plant environmental responses, gene regulatory networks, transcriptional networks, systems biology, plant defense

**Invited Speaker #37. Understanding the transcriptional regulation of the salt-stress response one cis-element at a time.** (Submission 388)
Jose Dinneny¹, Rui Wu¹, Shahram Emami¹
¹Carnegie Institution for Science, Department of Plant Biology, United States

Plant development is an intimate collaboration between the environment and genes. Our lab is interested in understanding how plants use environmental cues to determine the spatial and temporal control of transcriptional programs and how this regulation ultimately results in the patterning of tissues important for growth under specific environments. Salt stress responses result in highly tissue-specific and temporally dynamic transcriptional changes that cause growth to pause and recover during acclimation. Stress hormone signaling is also
differentially regulated in the root system with specific root types being hotspots for growth regulation. We have taken a synthetic biology approach to identify and functionally characterize the controlling DNA sequences in promoters that determine the spatial specificity of these transcriptional programs. These studies are identifying the essential regulatory elements plants use to control spatiotemporal transcriptional programs, which ultimately regulate growth and physiological adjustments to high salinity.

Keywords: Salt stress, transcriptional regulation, synthetic biology, cis-regulatory elements

Invited Speaker #38. Transcriptional feedback mechanisms that impact lignin biosynthesis in Arabidopsis (Submission 336)
Clint Chapple
Purdue University, United States
The products of the phenylpropanoid pathway range from complex, insoluble polymers such as lignin and suberin, to soluble flavonoids and hydroxycinnamate esters, to volatile compounds used to attract pollinators. In addition to its important role in plant biology, lignin has a significant impact on the effectiveness of converting cell wall polysaccharides to ethanol or second-generation biofuels. For this reason, a great deal of research has focused on altering phenylpropanoid metabolism through mutation or RNAi-mediated down-regulation of genes encoding pathway enzymes. Although many of these manipulations lead to significant alterations in plant metabolism, defects at a number of biosynthetic steps lead to a common suite of pleiotropic phenotypes such as dwarfing and sterility, the severity of which is dependent on the strength of the metabolic restriction.

We identified several semi-dominant mutants which contain lower levels of soluble phenylpropanoids including lignin and carry point mutations in the previously uncharacterized gene At2g48110. These mutants are also dwarfed and have a pale seed coat. As in several other mutants we have characterized, the decreased levels of the phenylpropanoid-derived UV protectant sinapoylmalate results in a reduced epidermal fluorescence (ref) phenotype, and we have named this mutant ref4. We have obtained multiple lines of additional supporting evidence that the REF4 protein play a role in the suppression of phenylpropanoid biosynthesis in wild-type plants. REF4 and its paralog, REF4-related 1 (RFR1), have recently been shown to be components of Mediator, a large multi-protein complex that facilitates interactions between DNA-bound transcription factors and RNA polymerase II. Mutants of Arabidopsis that lack REF4 and RFR1 hyperaccumulate phenylpropanoids, are viable and show little in the way of developmental changes. Surprisingly, ref4/rfr1 mutations mitigate the dwarf phenotype and sterility of the ref8 mutant of Arabidopsis which is defective in the early phenylpropanoid pathway enzyme p-coumaroyl shikimate 3-hydroxylase. Our data reveal that dwarfism in at least some phenylpropanoid pathway mutants is the result of a cascade of transcriptional misregulation that is dependent on Mediator.

Keywords: lignin, phenylpropanoid, bioenergy, Mediator

Invited Speaker #39. The topsy-turvy world of metabolic regulation (Submission 10)
Daniel Kliebenstein
University of California, Davis, United States
A central goal of systems biology is modelling how complex systems are regulated. One of the most complex systems in multi-cellular biology is metabolism which exists as a large non-cell autonomous network involving reactions in diverse cell types connected by the vascular system. This multi-cellular aspect complicates the ability to take regulatory models built on cell-autonomous systems and apply them to understand how metabolic pathways are controlled. We apply diverse genomics tools from genome wide association mapping to high-throughput Yeast-1-hybrid (Y1H) to expand our understanding of how plant metabolism is regulated. Using primary metabolism as detected by metabolomics, we have tested which genomes control the variation in plant metabolism and how this functions. Using reciprocal populations, we have shown extensive causal variation present within the organelles of Arabidopsis accessions. Organellar variation influences multiple levels of the plant metabolome beyond the standard phenotypes indicating that its influences systems beyond the organellar membranes. We also focus on the glucosinolates that controls Brassicales field fitness. Thus, this metabolic pathway and its inherent natural variation must be intricately regulated to properly respond to the biotic and abiotic environment within which that specific plant exists. A central question to this system is how the natural
variation in this metabolic pathway is coordinated within the regulatory network. Work will be presented showing that the end products of this pathway are actually key regulators of core physiological and transcriptional processes and that the plant must have a complement of structurally specific glucosinolate sensors. Another fundamental question we are addressing using secondary metabolites is to ask how many transcription factors can actually influence a metabolic pathway. Using a high-throughput Y1H approach, we have found that the aliphatic glucosinolate pathway likely has >100 TFs that control the accumulation of glucosinolates at a level consistent with dramatic field fitness consequences. These TFs predict key biotic and abiotic influences expected to modulate glucosinolate content in a complex environment. These inputs were also found using a novel genome wide association mapping approach. This is generating unexpected observations about how "secondary" metabolic pathways are as central in regulatory networks as primary systems.

Keywords: genome wide association, glucosinolate, regulation, metabolomics, systems biology, natural variation, fitness, ecology, evolution, networks, receptor

Invited Speaker #40. Targeted manipulation of gene activity in plants via CRISPR/Cas toolkit (Submission 312)
Jianfeng Li

1Harvard Medical School, United States

Targeted control of gene activity is key for elucidating and manipulating gene functions in plant basic research and crop engineering. The clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) technology is emerging as a powerful genome editing tool in diverse organisms, which utilizes an easily reprogrammable guide RNA (gRNA) to guide the targeting of Streptococcus pyogenes Cas9 endonuclease. Using Arabidopsis and Nicotiana benthamiana protoplasts as model cell systems, we explored the versatility of CRISPR/Cas and derived technologies and optimized their efficiencies for multiplex genome mutagenesis, homologous recombination and targeted transcriptional regulation. Plant protoplasts turned out to be a useful system for rapidly evaluating the performance of a given combination of gRNA/Cas9 or gRNA/Cas9-based transcriptional regulator at the genomic target, and may also allow one-step generation of homozygous mutant plant of target gene through protoplast regeneration. To facilitate the application of the CRISPR/Cas technology in Arabidopsis, we bioinformatically generated an Arabidopsis genome-wide resource of 1,466,718 unique gRNA target sites covering over 99% of nuclear protein-coding genes.

Keywords: CRISPR/Cas technology, Plant protoplasts, Targeted genome editing, Homologous recombination, Targeted transcriptional regulation

Invited Speaker #41. Genetically-encoded reporters for the visualization of abscisic acid distribution and concentration changes in Arabidopsis (Submission 8)
Rainer Waadt1, Kenichi Hitomi2, Noriyuki Nishimura3, Chiharu Hitomi2, Stephen R. Adams1, Elizabeth D. Getzoff2, Julian I. Schroeder1

1University of California San Diego, United States, 2The Scripps Research Institute, United States, 3National Institute of Agrobiological Sciences (NIAS), Japan

Abscisic acid (ABA) is a plant hormone that regulates plant growth and development and mediates abiotic stress responses. Direct cellular monitoring of dynamic ABA concentration changes in response to environmental cues is essential for understanding ABA action. We have developed ABAleons: ABA-specific optogenetic reporters that instantaneously convert the phytohormone-triggered interaction of ABA receptors with PP2C-type phosphatases to send a fluorescence resonance energy transfer (FRET) signal in response to ABA. ABAleons exhibit ABA affinities in the range of 100-600 nM and dynamic ranges of ~ 10 %. When expressed in Arabidopsis, ABAleons partially repress ABA responses. Despite these findings, ABAleons enable the mapping of ABA concentration differences in distinct cell types, time-resolved analyses of ABA uptake and transport from the hypocotyl to the shoot and root and the analyses of ABA concentration changes in response to environmental cues in guard cells and roots.

Keywords: plant hormones, abscisic acid, genetically-encoded reporters
Invited Speaker #42. Symplastic regulation of root patterning (Submission 338)
Shuang Wu¹, Christophe Perin², Kimberly Gallagher³
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Formation of specific cell types and tissues at defined times and positions is essential for the development of multicellular organisms. Often this developmental precision is achieved through the activity of signaling gradients that guide patterns of differential gene expression, the formation of developmental boundaries and ultimately cell fate specification. Here we examine the roles of the SHORT-ROOT (SHR) proteins from Arabidopsis thaliana, Brachypodium distachyon and Oryza sativa, in root ground tissue patterning. In A. thaliana, SHR is a plasmodesmata-dependent mobile signal that plays a concentration dependent roll in root patterning. Our results with the SHR homologs from distachyon and O sativa suggest that the extent of SHR movement directly determines the number of cortex layers formed in these roots. Our recent work with a tissue specific inhibitor of plasmodesmata-dependent movement suggests that SHR independent pathways may limit the number of cortex cell layers in A. thaliana

Keywords: development, signaling, plasmodesmata

Invited Speaker #43. What factors determine the positioning of meristematic zones in leaves? (Submission 228)
Hirokazu Tsukaya¹
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In angiosperms, the meristematic zone of the leaf primordia (leaf meristem) is located at the base of the leaf in the rice family, and at the junction between the leaf blade and petiole in Arabidopsis. This positioning of the meristematic zone in primordia of simple leaf types is opposite to that in shoot apical meristems (SAM). However, the meristematic zone in the primordia of fern leaves is located in the margins or apices, similar to SAM. We also found that in some gymnosperms, such as Gingko biloba, the leaf meristem is located at the margins of primordia. Interestingly, even in Arabidopsis, the meristematic zone of petal primordia is located in the apical margin. Moreover, compound leaves are classified into two major types: the acropetal type, in which leaflets develop from the base to the apex along the longitudinal axis of the leaf primordia, and the basipetal type, in which leaflets develop from the apex to the base. Therefore, the position of the meristem in the leaf primordia varies among species, leading us to question what genetic changes are involved in the reverse positioning of the meristematic zone from the apices to the bases.

Our comparative analyses of the direction of organogenesis in two species of compound leaves in Papaveraceae revealed that gradient expression of the CINCINNATA homolog could explain the directional organogenesis in one species but not in the other species, indicating that the directionality of leaflet initiation could have diversified via different mechanisms in a case-by-case manner. If so, what is the key regulator behind leaf meristem positioning in the simple leaf type of Arabidopsis? Previously, we reported that a phylloclade of Asparagus asparagoides (monocot), a simple-leaf-like metamorph of the lateral shoot that ectopically expresses some leaf genes, has a meristematic zone at its base. This led us to speculate that certain "leaf programs" involve a shift in the meristematic zone from the apex to the base of the primordia. What factors of the leaf programs are involved in the longitudinal positioning of the leaf meristem?

Recently, we focused on ANGUSTIFOLIA3 (AN3) and ROT4-Like (RTFL) family peptides as potential regulator candidates for leaf meristem positioning in Arabidopsis. Our present understanding of the possible roles of these factors in determining leaf meristem position will be discussed here.


Keywords: leaf primordia, meristematic zone, positioning, ANGUSTIFOLIA3, RTFL

Invited Speaker #44. Early signaling in plant stress response (Submission 320)
Sorina Popescu¹
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Cellular signaling governs basic cell functions including the detection of pathogens and other environmental stressors. In my lab, we study stress-triggered signaling pathways in several plant models. We employ large-scale
approaches to obtain information on proteins, such as their interactions with other molecules or chemical modifications, and assemble data in signaling networks. Individual network components are then studied using classical, low-throughput molecular biology and biochemistry tools. This hybrid strategy helps us understand the overall architecture of cellular signaling pathways and how discrete errors in this system impact signal transmission and cellular responses. I will present recent results of several lab projects, including a study of signaling pathways at the confluence of biotic and abiotic stress response and a study of the mechanisms of pathogen resistance in plants focused on the contribution of kinase-mediated signaling in diverse cellular processes activated by pathogen infection.

Keywords: Kinase signaling, abiotic stress, biotic stress, signaling networks

Invited Speaker #45. Molecular mechanisms underlying the dynamics of the plant steroid receptor activation (Submission 286)
Mathilde L.A. Simon¹, Matthieu P. Platre¹, Yvon Jaillais¹
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In plants, receptor kinases form the largest family of membrane receptors and they are involved in virtually all aspects of the plant life, including development, immunity and reproduction. Although a few model receptor kinases have been studied extensively, there are still many with unknown function. Besides, we still know very little on how those receptors are activated. The plant growth hormone Brassinosteroid is perceived at the plasma membrane by BRI1, a Leucine-Rich-Repeat receptor kinase. Here we address the molecular mechanisms of BRI1 activation. We report that the BRI1 KINASE INHIBITOR 1 (BKI1) is tyrosine phosphorylated in response to brassinosteroid perception. Phosphorylation occurs within a reiterated [KR][KR] membrane targeting motif, releasing BKI1 into the cytosol and enabling formation of an active signaling complex between BRI1 and its co-receptor BAK1. We will discuss the molecular bases of BKI1 membrane targeting and their implication for brassinosteroid signaling.

Keywords: Receptor kinase signaling, Brassinosteroids, Membrane biology, Cell biology, Arabidopsis, Protein phosphorylation

Invited Speaker #46. Signatures of polygenic adaptation in the Arabidopsis genus (Submission 420)
Juliette de Meaux¹, Fei He¹, Agustin Arce¹, Martin Lercher², Ulrich Wittelsbuerger²
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For a mature understanding of the molecular basis of adaptation, methods are required that include mutations of all effect sizes. Thus, to unravel the true polygenic underpinnings of adaptation in the genus Arabidopsis, the genomic cartography of functional mutations is required in each lineage. Genes are expressed as a result of the complex interaction between cis-regulatory DNA located within, or around, the expressed locus with trans-acting element(s) encoded elsewhere in the genome. So far, the most insightful analyses of polygenic adaptation, conducted in yeast, was based on the distribution of cis-regulatory variants. We have completed a genome-wide screen of cis-regulatory variants derived in the two sister species A. lyrata and A. halleri and will show that this approach can point to novel molecular systems recently optimized by natural selection.

Keywords: genetic adaptation, drought, heavy metal tolerance, Arabidopsis genus, cis-regulatory variation

Invited Speaker #47. Predicting evolutionary dynamics of seasonal adaptation to novel climates in Arabidopsis thaliana (Submission 487)
Alexandre Fournier-Level¹, Johanna Schmitt²
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Whether and how populations will adapt fast enough to persist in the face of rapid climate change is a critical question for plant biologists. To examine the genetic basis of life history traits and predict their evolution in novel seasonal climates, we grew a core collection of MAGIC lines of Arabidopsis thaliana in growth chambers programmed to simulate four different climate scenarios. In each climate treatment, we exposed successive seasonal cohorts to daily and seasonal temperature fluctuations in real time through a simulated year, and recorded growth traits, reproductive phenology, fecundity, and seed germination for plants of each genotype.
Using these data, we developed a linear mixed model of trait effects on fitness, using genomic selection methods to predict trait phenotypes and fitness for each genotype in each seasonal environment. We used this model to simulate replicated evolutionary trajectories of the same base population over 50 years with moderate outcrossing in each climate scenario, as well as scenarios of abrupt or gradual climate change from a reference climate. Life history traits evolved and fitness increased in all scenarios, but the pattern and extent of adaptation differed across climates and seasons. The simulations showed a sharp initial decline in genetic diversity as the base population adapted to novel conditions, followed in some cases by recovery of diversity as recombination generated new genotypes as substrates for selection. The genetic architecture of selection response differed among climatic scenarios, resulting in qualitative differences in the dynamics of adaptation. Initial adaptation to a reference climate reduced the evolutionary potential for subsequent adaptation to climate change. Genomic selection methods combined with simulation of evolutionary trajectories make it possible to explore the dynamics of phenotypic and genotypic evolution in replicated scenarios of climate change.

Keywords: adaptation, climate change, genomic selection, flowering time, natural variation

Invited Speaker #48. Molecular mechanism and physiological function of cytoplasmic streaming (Submission 314)
Motoki Tominaga1
1RIKEN, Japan
In contrast to animal which can move freely, plant cannot move all through life at a place it germinated. However, in plant cell, we can observe active intracellular movement called “cytoplasmic streaming” which was reported first in 1774 by Bonaventura Corti. Cytoplasmic streaming is generated by organelle associated plant specific myosin (class 11) moving along actin bundles. Because cytoplasmic streaming is widely occurring in plant cells ranging from algae to angiosperms, it is supposed to be an essential system for plant. Molecular mechanism and physiological function of cytoplasmic streaming have remained unclear since its discovery more than 200 years ago, although it has been widely studied in the field of plant science. I have revealed function of cytoplasmic streaming both at molecular- and higher-level. First, I have characterized morphological and mechanical, enzymatic properties of a higher plant myosin 11 purified from tobacco BY-2 cells. Sequence analysis and electron microscopic observation revealed that tobacco myosin 11 forms dimer and has two head domains at the N-terminus, each attached to a long neck domain, and two globular tail domains at the C-terminus of tail domain. An optical trap nanometry revealed that single myosin 11 molecule moves processively along actin with 35 nm steps at 7 um/sec, the fastest among known processive motors. These results suggest that myosin 11 function is optimized for transporting organelle with small numbers of molecules at high velocity (Tominaga et al., EMBO J, 2003; Tominaga et al., JBC, 2012; Tominaga and Nakano, Front. Plant Sci., 2012). Second, I have revealed physiological function of cytoplasmic streaming by controlling myosin 11 velocity artificially. I generated high- and low-speed chimeric myosin 11 by replacing the motor domains of Arabidopsis thaliana myosin 11-2 with those of Chara coralline myosin 11 and Homo sapiens myosin 5b, respectively. Surprisingly, the plant sizes of the transgenic Arabidopsis expressing high- and low-speed chimeric myosin 11-2 were larger and smaller, respectively, than that of the wild-type plant. This size change correlated with acceleration and deceleration, respectively, of cytoplasmic streaming. These results strongly suggest that cytoplasmic streaming is a key determinant of plant size (Tominaga et al., Dev. Cell, 2013). I would like to discuss about a fundamental function of cytoplasmic streaming in plant including molecular mechanism.

Keywords: Cytoplasmic streaming, Myosin, Plant size

Invited Speaker #49. Plant Synthetic Biology (Submission 498)
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Our work focuses on plant synthetic biology, a research field that combines approaches from engineering and biology. Synthetic biology seeks to advance biology in a way similar to what electronics did in the 20th century. For example, in electronics an integrated circuit is designed once and can be reused in diverse devices such as cell phones, computers, or aircraft. By designing integrated gene circuits in plants we can produce new functions
such as a quantitatively tuned drought resistance gene that provides a level of response proportional to the drought. To produce the highly predictable function, synthetic biology uses mathematical analyses, modeling and standardized orthogonal parts. We have used synthetic biology to develop entirely new tools and possibilities in plants. For example, we have enabled a computerized detection technology in plants using computationally redesigned proteins that signal via a synthetic signal transduction system. In the presence of the ligand a high energy histidine kinase signal is transmitted to a synthetic protein that activates transcription. By linking the computational input to a synthetic degreening circuit, we have produced plants capable of detecting and responding to ligands of interest. In addition, we have produced a simple logic gate, NOT gate and quantitatively characterized synthetic gene components that provide a high degree of cooperativity. These gene parts can be assembled to produce NOR gates, which are Boolean complete, allowing computer-like logic gene circuits in plants. The synthetic gene circuits provide the ability to design electronic-like controls such as switches, logic gates and memory for plant function. In designing such synthetic gene circuits we are able to dissect natural processes and further understand the complexity of plant systems.

Keywords: Synthetic biology, Synthetic signal transduction, Gene circuits

Invited Speaker #50. Synthetic biology in photosynthetic organisms: Redirecting reducing power (Submission 278)
Poul Erik Jensen

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Oxyngeic photosynthesis converts solar energy into chemical energy that is used to create carbon building blocks required for synthesis of a wealth of chemical compounds. Many plants also produce bioactive natural products which may have potential uses as pharmaceuticals [1]. In the course of evolution, energy generation and biosynthetic capacities have become compartmentalized. Photosynthesis, located in the chloroplasts, provides ATP and NADPH as well as carbon sources for synthesis of carbohydrates, amino acids and nucleotides. The bioactive natural products are being synthesized in the endoplasmic reticulum (ER). Especially one group of enzymes is involved in the synthesis of a wide spectrum of bioactive natural products. These are the cytochrome P450 monoxygenases (P450s) which are powered by single electron transfers from NADPH via a reductase. P450s are often present in low amounts and the reactions proceed relatively slow due to limiting concentrations of NADPH. Through a synthetic biology approach, enzymes like the P450s can be coupled directly to the photosynthetic energy generation to obtain environmentally friendly production of complex chemical compounds. We have demonstrated that it is possible to break the evolutionary compartmentalization of energy generation and P450-catalysed biosynthesis, by relocating an entire P450 dependent pathway, producing the bioactive compound dhurrin, to the chloroplast and driving the pathway by direct use of the reducing power generated by the photosynthetic apparatus [2]. This demonstrates the potential of transferring pathways for structurally complex natural products to the chloroplast and directly tapping into the reducing power generated by photosynthesis to drive the P450s using water as the primary electron donor. Current work is directed towards establishing stable tobacco plant lines and cyanobacteria with multi-enzyme pathways expressed in the proximity of the photosystems able to produce commercially interesting bioactive compounds directly driven by light.


Keywords: synthetic biology, photosynthesis, bioactive compounds, light-driven

Keynote Invited Speaker #51. Arabidopsis and Plant Hormones: The evolution of the revolution (Submission 378)
Peter McCourt

University of Toronto, Canada

The central role of plant hormones in growth and development has always been obvious to any one who took a plant biology course. However, 30 years ago when I was a graduate student lectures mostly involved questioning
the logic of hormone action rather than asking logical questions. Hormones were cataloged by the physiological changes they caused or by how changes in physiology affected their levels. These endless descriptions of hormone action in a myriad of plants encouraged one wag to tell me "stay away from that hormone morass if you want to get a job". This all changed with the advent of modern Arabidopsis research in the 1980s. It is now clear how Arabidopsis genetics turned hormones research from anecdotal phenomena into defined pathways of synthesis and signaling and I will point out some of these themes. However, although we now hold these genetic truths to be self-evident, the revolution is not over. New approaches led by Arabidopsis research involving systems and chemical biology are transforming hormone-signaling from linear highways to subway networks. I will touch on these using examples involving an old hormone Abscisic Acid (ABA) and relatively new one, Strigolactones (SL). Finally, although plant hormones are inherently interesting at a basic biological level, historically, they have been important targets of Ag-biotechnology. In this pursuit, the Arabidopsis revolution is again defining new lead genes for biotech application. This little weed that many have said "is not really a plant" will again be required if we are to make logical decisions on how to make the plants we want.

Keywords: Plant hormones, Translational plant biology, ABA, Strigolactones

Keynote Invited Speaker #52. From phenotypes to pathways: global exploration of cellular networks using yeast functional genomics (Submission 491)
Brenda J Andrews
The Donnelly Centre, University of Toronto, Canada
The entire landscape of eukaryotic genetic research has been transformed by our ability to rapidly sequence genomes - while we can now map genomes efficiently, we do not yet know how to interpret genome variation to predict inherited phenotypes. Emerging evidence suggests that we must account for genetic interactions in order to relate genotype to important phenotypes in any eukaryotic system. To systematically explore genetic interactions, our group developed a unique functional genomics platform called ‘synthetic genetic array’ (SGA) analysis that automates yeast genetics and enables the systematic construction of double mutants. We developed two powerful pipelines which combine SGA and automated microscopy for systematic and quantitative cell biological screens or phenomics. Our first pipeline uses SGA to introduce fluorescent markers of key cellular compartments, along with sensitizing mutations, into yeast mutant collections. We then perform live cell imaging on the mutant arrays using HTP confocal microscopy to quantitatively assess the abundance and localization of our fluorescent reporters, providing cell biological readouts of specific pathways and cellular structures in response to thousands of genetic perturbations. Our second pipeline exploits the yeast GFP collection, a unique resource consisting of thousands of strains with different genes uniquely tagged with GFP. This remarkable collection has been arguably underutilized for systematic analysis of the proteome, largely due to the challenges associated with analysis of large sets of cell biological data. We addressed this challenge by adopting a high-content screening approach to measure protein abundance and localization changes in an automated fashion on a genome scale. Our general approach, in particular our network analysis and visualization methods, are readily extensible to other systems.

Keywords: synthetic genetic array (SGA) analysis, yeast genetics, genetic interactions, phenomics, yeast GFP collection

Biotechnology, Bioenergy & Food Security

Poster/Talk #53. Global Marketing Software Connecting Local Foods Socially (Submission 97)
Rajnish Khanna, Robert Muller, Lynn Monica, John Allen, Pamela Ronald, Harold McGee, Winslow Briggs, Wolf B Frommer
Global Food Scholar, United States
Imagine a local food system with transparency of real time links between food growers, sellers and consumers. At first look, this is nearly impossible to achieve. The current technology infrastructure is capable of supporting farm-to-fork databases. The challenge is to gather real time information. A group of plant biologists and software
engineers have gotten together in the Silicon Valley to solve this problem and transform food system networks. Visibility within the food networks will cause a major shift towards food safety and security. Now imagine carrying a local food network map on your digital device and discovering what's locally grown for breakfast in Portland, lunch in San Francisco, and supper in New York. Make reservations, pre-order from the local menu, or act local anywhere by accessing all the local food and beverage markets across the globe. It's a tall order, but we have embarked on our journey to meet this challenge by creating new marketing technologies to enhance farmer-to-consumer connectivity. Global Food Scholar, Inc. is developing a mobile application to enable discovery of local and specialty foods, provide invaluable marketing support to beginning and disadvantaged farmers, improve grower outreach, and strengthen food system infrastructure, including local farm to school programs. The mobile technology will promote small sized breeding programs involved in developing novel traits in specialty crops by branding and creating new marketing outlets. A Pilot Project in the Bay Area, CA is creating unprecedented networks of food producers, sellers (markets and restaurants) and consumers. The mobile application will be launched in the Bay Area in June/July 2014. The software will be available free to improve growers’ profitability and consumers’ diets.

Keywords: healthy food, local food, farm to table, apps

Poster/Talk #54. A rapid method for translating molecular tools for crop species (Submission 131)

Mily Ron1, Kaisa Kajala1, Germain Pauluzzi2, Dongxue Wang3, Mauricio A. Reynoso2, Kristina Zumstein1, Jasmine Garcha1, Sonja Winte1, Helen Masson1, Soichi Inagaki1, Fernan Federici5, Neelima Sinha1, Roger Deal3, Julia Bailey-Serres2, Siobhan Brady1

1UC DAVIS, United States, 2UC Riverside, United States, 3Emory University, United States

Root development has been characterized at great spatiotemporal resolution in model plants including Arabidopsis and rice. Translating existing molecular tools into crop species is fundamentally important for expanding our understanding of plant development and stress responses. However, generating transgenic lines of non-model species can be labour-intensive and take a long time. We have used Agrobacterium rhizogenes-mediated hairy root transformation to rapidly generate transgenic roots in order to optimize a range of molecular tools in tomato.

We assessed reporter lines expression patterns in tomato hairy roots, showing that the expression patterns in A. rhizogenes-transformed hairy roots are indistinguishable from roots transformed with A. tumefaciens and generating a toolbox of cell- and tissue-type specific promoters for tomato roots. We optimized two molecular methods for cell-type specific gene expression studies in tomato: Isolation of Nuclei Tagged in specific Cell-Types (INTACT) and Translating Ribosome Affinity Purification (TRAP). Furthermore, transcriptional reporters, translational reporters and CRISPR/Cas9 genome editing suggest that SHORT-ROOT and SCARECROW gene function is conserved between Arabidopsis and tomato. Finally, we have also used the hairy root method to generate transgenic roots in wild tomato Solanum pennellii as well as Lepidium hyssopifolium and Arabidopsis.

Keywords: Roots, Hairy root transformation, Cell type-specific profiling, genome editing, tomato

Poster/Talk #55. A novel density-based screen for mutants with altered seed oil (Submission 165)

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Seed oils from Brassicaceae are becoming increasingly important as industrial and biofuel feedstocks, and improvements in oil content will greatly enhance their potential to meet this demand. In order to understand the genetic pathways that control oil production, we are focused on identifying new candidate genes involved in these processes in Arabidopsis, and transferring this new knowledge to the emerging oilseed crop Camelina sativa. It has previously been shown that seeds with altered oil content also have altered density, and that this can be used to isolate high and low oil mutant lines from mutagenised populations (Focks and Benning, 1998; Shen et al., 2006). However, as these screens utilise toxic organic compounds for separation, we developed an alternative
screen employing calcium chloride solutions optimised using previously characterised high and low oil mutants. Seed pools from several publically available activation tagged collections were screened using a high concentration calcium chloride solution to isolate high density (putative low oil) seeds, followed by a low concentration calcium chloride solution to isolate low density (putative high oil) seeds. The putative low and high oil seeds (1,929 and 3,157 lines respectively) were regrown and oil content was measured for each line using gas chromatography (GC). During GC screening, several seed mutants were identified based on visible characteristics (seed colour and morphology) that are known to be associated with altered oil content. In addition to characterising these lines, we are currently analysing candidate mutants selected based on altered oil content. We anticipate that the candidate lines will reveal novel genes involved in oil biosynthesis in Arabidopsis.

Keywords: seed oil, Brassicaceae, mutant screen

**Poster/Talk #56. Analysis of Leaf Proteome to Determine Possible Cross-Tolerance to Abiotic Stresses in Soybean Cultivars (Submission 183)**

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Soybean (Glycine max (L.) Merr.) is a legume that provides a significant source of proteins and fatty acids in both human and animal diets. High temperature stress (HS) and water stress (WS) during cropping season are the two most limiting abiotic stress factors affecting crop growth and yield. Water deficit is syndrome that affects several processes including denaturation of proteins, and cellular membranes. Heat stress, also affects several processes including photosynthesis and respiration and fertility in plants. Combination of these two factors may lead more negative impacts on plants than either Cultivars may differential responses to abiotic stresses and obtaining insights into the effects of WS and HS on molecular and cellular functions of soybean will provide information for plant breeding. An experiment was conducted by varying WS and HS either alone or in combination in two soybean cultivars which are WS and HS tolerant and susceptible. Changes in leaf protein composition were studied by 2-DE Gel electrophoresis complemented with Mass spectrometry. Plant biomass and physiological parameters along with stress levels were recorded. PD Quest analysis revealed at least 200 proteins in both cultivars, of which, 61 proteins were differentially expressed in response to WS and HS. Differentially expressed leaf proteins were excised from 2-DE gel. The peptide sequence tags generated from the spots were queried through MASCOT search. Gene ontology analysis for each protein was identified for functional categories including photosynthesis, metabolism, transport, stress and defense, and glycolysis. The majorities of heat responsive-proteins were up regulated during heat stress and combined stress in cultivar susceptible cultivar; these proteins were down regulated under to water stress conditions. However in tolerant cultivar, the heat shock proteins were generally down regulated to all levels stress. This study reveals the differentially expressed proteins in two contrast genotypes and their possible role in drought tolerance, as well as the possibility of the plant's development of cross-stress tolerance. Our studies showed that differentially expressed proteins involved in antioxidant defense were mostly up-regulated, whereas proteins associated with photosynthesis, secondary metabolism, and amino acid and protein biosynthesis were down-regulated in response to heat stress.

Keywords: Abiotic stress, Cross-tolerance, Soybean, Proteomics

**Poster/Talk #57. Directed Minichromosome Engineering via Haploid Induction (Submission 239)**

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Haploid induction (HI) is the cornerstone in doubled haploid plant breeding programs and is currently the fastest way to breed desirable traits into crops. We have previously reported an efficient HI strategy using altered versions of the centromere-specific histone CENH3, in which the chromosomes marked with altered CENH3 are eliminated when crossed to wild type. Using Illumina sequencing, we performed chromosome dosage analysis on the haploid progeny resulting from the HI cross and observed a low frequency of centromere-containing minichromosomes (minis) derived from the HI genome. Minis are extremely useful because they are usually
transmitted well to the progeny but are non-essential and have the potential to confer new traits into existing lines without linkage drag. To show that our methods can robustly create minis of different sizes, we generated selectable minis from all five Arabidopsis chromosomes. Bioinformatics and cytogenetic analysis of the breakpoints of one of our Chr1 minis show that it forms a ring and that it is meiotically and sexually transmitted over multiple generations. To exploit the full potential of minis, we are taking advantage of random dsDNA breaks that occur during HI or targeted dsDNA breaks induced through CRISPR-Cas9 for further manipulation of minis in planta. Minis that can be engineered and transferred concurrently through HI could accelerate both functional genomics and trait introgression for plant breeding.

Keywords: haploid induction, minichromosomes, genome engineering

**Poster #58. Biotechnological Improvement of Plant Biomass for desired applications** (Submission 273)
Hyung-Woo Jeon¹, Jin-Seong Cho², Young-Im Choi³, Jae-Soon Lee³, Mi Kwon⁴, Jae-Heung Ko¹
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Woody biomass has gained a huge research interests as a potential renewable resource for liquid biofuel production. To improve woody biomass production, it is necessary to increase the trees’ growth rates and stem volumes. Gibberellins are phytohormones that regulate many developmental aspects of plant including stem growth. Here, we produced transgenic Arabidopsis and poplar expressing GA20-oxidase, a key enzyme of the GA biosynthesis pathway, from Pinus densiflora under the control of either CaMV 35S or developing xylem-specific (DX) promoter. As we expected, both transgenic Arabidopsis showed accelerated stem growth and increased biomass compared to WT. In addition, increased secondary wall thickening and elongation of fiber cells were manifested. However, no significant differences were found between 35S and DX specific expression of PdGA20ox in Arabidopsis. Both transgenic poplar trees also showed dramatic increase of biomass with accelerated stem growth. Furthermore, xylem differentiation was enhanced by increased xylem cell pile numbers up to 200% compared to control plant. However, in 35S::PdGA20ox poplar trees, some undesirable phenotypes, such as poor root growth with smaller leaf development, were accompanied. Interestingly, transgenic poplar expressing PdGA20ox under the control of DX promoter resulted in much reduced undesirable phenotypes. Further characterization of both transgenic Arabidopsis and poplar plants will be presented. This work was funded by a grant from the Korea Forest Service (S111213L080110).

Keywords: Gibberellin 20-oxidase, developing xylem, woody biomass

**Poster #59. Translating frost tolerant seed degreening from Arabidopsis to Canola** (Submission 417)
Mendel Perkins¹, Subramanian Sankaranarayanan¹, Srijani Deb¹, Gayathri Wewala¹, Marcus Samuel¹
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Non-lethal frost during key stages of Brassica napus (canola) embryo development is known to significantly increase canola seed chlorophyll levels. High green seed content reduces canola pricing causing significant economic losses to canola producers. Components of the seed de-greening pathway have been identified in the model species Arabidopsis thaliana. ABI3, a transcription factor implicated in mediating abscisic acid (ABA) responses has been shown using a microarray to regulate a suite of genes largely relating to seed maturation and de-greening. Specifically, during seed maturation ABI3 is required for the transcriptional activation of the downstream SGR2 (stay-green) gene by binding to the SGR2 promoter, to promote degreening. In Arabidopsis, overexpression of ABI3 was sufficient to impart frost-tolerant degreening. Given the high level of sequence similarity of SGR2 and ABI3 between Arabidopsis and Brassica, it is expected that the system can be efficiently translated into Canola. To achieve this, Brassica napus homologs of SGR2 and ABI3 were isolated and tested for their ability to perform similar functions through complementation using a suite of Arabidopsis mutants. In parallel, canola transgenics that hyperaccumulate ABI3 under stress conditions have been developed. These transgenics are being currently evaluated for their ability to confer both frost tolerance and frost-tolerant degreening.

Keywords: Canola, degreening, frost tolerance, abscisic acid
Biotic Responses/ Plant Defense

Poster/Talk #60. Understanding Quantitative Resistance through Natural Variation in both Botrytis cinerea and Arabidopsis thaliana (Submission 9)
Jason Corwin¹, Susanna Atwell¹, Daniel Kliebenstein¹
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Our current understanding of disease resistance in natural plant populations is largely framed by studies analyzing large-effect, qualitative resistance loci controlled by presence/absence of R-genes, including RLKs and NBS-LRRs. However, many plant-pathogen interactions are quantitative in nature without any large effect qualitative loci. To identify the genes controlling quantitative resistance, we conducted a genome-wide association (GWA) study using 96 accessions of A. thaliana and 4 phenotypically distinct isolates of B. cinerea. Infected leaves were monitored at 72 hpi for 86 visual phenotypes and 45 chemical phenotypes using semi-automated image analysis and HPLC assays. Significant effects for the host, pathogen, and their interaction were identified for 86%, 70%, and 51% of the phenotypes using a multivariate ANOVA. For most phenotypes, the genetic background of the pathogen controlled a larger proportion of the variance than the plant. To map the phenotypic effect of individual genes in the host, a heteroscedastic effects model was applied to a collection of 115,301 SNPs (MAF > 0.2) present among the Arabidopsis accessions for biologically relevant phenotypes. Lesion area identified a total of 3,354 Arabidopsis genes that associated to phenotypic variation in resistance to the isolates with only 14% of those genes identified in 3 or more isolates indicating that quantitative resistance is highly isolate specific. Further, while we do see enrichment in RLKs and NBS-LRRs, this only accounts for 0.03% of the total genes and the majority of identified genes have not been previously associated with disease resistance. The genes associated with lesion size were filtered into co-expression networks using the ATTEDII database and condensed into 246 networks larger than 10 genes. The largest of these networks involve a variety of known biological functions including development, vesicle trafficking, disease resistance, lipid metabolism, cell death, protein turn-over, and cell wall metabolism. Individual genes within the largest networks were selected for validation using T-DNA insert lines resulting in novel host genetic associations with quantitative resistance. Our results illustrate the complexity and diversity of biological functions associated with disease resistance, identifies the components of disease resistance with natural genetic variation, and generates new hypotheses to explore with regard to quantitative disease resistance.

Keywords: Quantitative resistance, Association mapping, Botrytis cinerea, Co-expression networks

Poster #61. SNC1-mediated immunity is negatively regulated by a chromatin remodeler (Submission 17)
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SNC1 (SUPPRESSOR OF NPR1, CONSTITUTIVE 1) is one of a suite of intracellular Arabidopsis NOD-like receptor (NLR) proteins which induce defense responses upon detection of pathogenic molecules within the host cell. However, the molecular mechanisms that lead from NLR protein activation to immune response induction have only been partially characterized. To identify negative regulators of NLR protein-mediated immunity, a forward genetic screen was undertaken to look for enhancers of the dwarf, autoimmune gain-of-function snc1 mutant. To avoid potential lethality resulting from severe dwarfism the screen was conducted using snc1 mos4 plants, which display wild type-like morphology and resistance, and the M2 progeny were screened for a reversion back to snc1-like phenotypes. One isolated mutant line, termed muse9, contains a molecular lesion in a chromatin remodeler. The muse9 snc1 mos4 triple mutant is dwarf and displays elevated expression of the pPR2::GUS reporter gene, although resistance to the virulent pathogen Hyaloperonospora arabidopsidis Noco2 is between snc1-like and wild type-like. Similarly, the muse9 single mutant has a subtle morphological phenotype but no observable alteration from wild type-like resistance. Transcription of SNC1 is significantly increased in muse9 as compared to wild type and in the muse9 snc1 double mutant as compared to snc1. These data suggest that MUSE9 plays a subtle but specific role in the regulation of SNC1 expression and SNC1-mediated immunity. MUSE9 may work together with other proteins at the chromatin level to repress SNC1 transcription. Such regulation is important for fine-tuning the expression of NLR genes to prevent autoimmunity.
Poster #62. AQUAPORIN-INTERACTING1 (AQI1), a new player in cell death regulation (Submission 35)
Eva Glink1, Tanja Hoch1, Karla Fischer1, Benjamin Neuhauser1, Uwe Ludewig1, Artur Pfitzner1
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Aquaporins (AQPs) are integral membrane water channels in animals and plants. Recent evidence suggests that AQPs are also channels for the cell death signal hydrogen peroxide. We have shown that transgenic overexpression of BHRF1, a protein from Epstein Barr virus, renders tobacco plants hypersensitive towards cell death signals elicited by pathogen infection and during senescence. Yeast two-hybrid (Y2H) analysis revealed that BHRF1 targets AQPs, indicating that BHRF1 may disturb cell death signaling through AQPs. In a screen for cellular regulators of AQP function, we identified Arabidopsis AQP-INTERACTING1 (AQI1). AQI1 is highly conserved in plants and animals. It can suppress cell death caused by transient overexpression of AQP fusion proteins in N. benthamiana. Furthermore, AQI1 is able to inhibit AQP-mediated water flux into frog oocytes, suggesting that AQI1 exerts its anti-cell death function by blocking the pores of AQPs. Indeed, Y2H studies showed that AQI1 binds in the pore-forming region of AQPs. In tobacco, AQI1 accumulates preferentially in young leaves, while it is degraded in older leaves. In summary, our data suggest that flux of cell death signals through AQPs is controlled by AQI1 acting as a plug on AQPs, and that the function of AQI1 is to protect juvenile tissue from premature ageing.

Keywords: aquaporin, hydrogen peroxide, pathogen defense, protein degradation, senescence

Poster #63. The Arabidopsis triphosphate tunnel metalloenzyme, AtTTM2, is a negative regulator of the salicylic acid-mediated feedback amplification loop for defense responses (Submission 37)
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The triphosphate tunnel metalloenzyme (TTM) superfamily represents a group of enzymes that are characterized by their ability to hydrolyze a range of tripolyphosphate substrates. Arabidopsis, encodes three TTM genes, AtTTM1, 2 and 3. Despite its annotation as adenylate cyclase, we found that AtTTM2 did not produce cyclic AMP, but rather exhibited pyrophosphatase activity. AtTTM2 knockout (KO) mutant plants exhibit an enhanced hypersensitive response, elevated pathogen resistance against both virulent and avirulent pathogens, and elevated accumulation of salicylic acid (SA) upon infection. In addition, stronger systemic acquired resistance was also observed. These enhanced defense responses are dependent on SA, PAD4, and NPR1. Despite their enhanced pathogen resistance, ttm2 plants did not display constitutively active defense responses, suggesting that AtTTM2 is not a conventional negative regulator, but a negative regulator of the amplification of defense responses. The transcriptional suppression of AtTTM2 during pathogen infection or treatment with SA and the SAR activator, BTH, further supports this notion. Such transcriptional regulation is conserved among TTM2 orthologues in the crop plants, soybean and canola, suggesting that TTM2 is involved in immunity in a wide variety of plant species. This indicates the possible usage of TTM2 KO mutants for agricultural application to generate pathogen resistant crop plants.

Keywords: disease resistance, pathogen, pyrophosphatase, salicylic acid, triphosphate tunnel metalloenzyme

Poster/Talk #64. PATTERNS AND RECEPTORS IN PLANT INNATE IMMUNITY (Submission 40)
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Host pattern recognition receptor-mediated perception of microbe-associated molecular patterns (MAMP) is a prerequisite for the initiation of antimicrobial defenses in all multicellular organisms including plants. As metazoans, plants have evolved immune receptors for the recognition of bacterial lipopolysaccharides, flagellin and peptidoglycan. Here, we will report on the identification of a plant peptidoglycan receptor complex mediating peptidoglycan sensing and immunity to bacterial infection, and will discuss convergent evolution of peptidoglycan recognition receptors across lineage borders. It is hitherto unknown, how complex bacterial structures, such as PGN, are perceived by plant pattern recognition
receptors (PRR) and whether host hydrolytic activities facilitate decomposition of bacterial matrices and generation of soluble PRR ligands. Here, we show that Arabidopsis upon bacterial infection or exposure to microbial patterns produces a metazoan lysozyme-like hydrolase (lysozyme 1, LYS1). LYS1 activity releases soluble PGN fragments from insoluble bacterial cell walls and cleavage products were able to trigger responses typically associated with plant immunity. Importantly, LYS1 mutant genotypes exhibited super-susceptibility to bacterial infections similar to that observed on PGN receptor mutants. We propose that plants employ hydrolytic activities for the decomposition of complex bacterial structures, and that soluble pattern generation might aid PRR-mediated immune activation in cell layers adjacent to infection sites. Further, identification of a novel pattern recognition receptor by exploiting the natural variation within Arabidopsis ecotypes will be reported. We have identified two novel pattern recognition receptors, RLP23 and RLP32, respectively, that contribute to immunity to microbial infection by sensing proteinaceous patterns from oomycete or bacterial microbes respectively. Both receptors belong to the class of leucine rich-repeat (LRR) proteins lacking a cytoplasmic konase domain. Both receptors require SOBIR1 as a co-receptor. New insights into LRR-P receptor signaling and the potential of pattern recognition receptors for engineering resistance in crops (PRR stacking) will be discussed.

Keywords: plant innate immunity, pattern recognition receptor, engineering disease resistance

Poster #65. Structure-function dissection of EDS1 disease resistance signaling (Submission 47)
Jane Parker1, Deepak Bhandari1, Clementine Le Roux1, Karsten Niefind2, Laurent Deslandes3
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In angiosperm plants, the nucleo-cytoplasmic lipase-like protein EDS1 signals with its sequence-related partners, PAD4 and SAG101, to confer basal and effector-triggered immunity (ETI) against host-adapted biotrophic pathogens. Many intracellular nucleotide-binding/leucine-rich-repeat (NLR) receptors, recognizing different pathogen effectors, converge on the EDS1 node for defense and programmed cell death. Nuclear accumulation of EDS1 and a number of EDS1-requiring TNL (Toll-Interleukin1-Receptor domain-NLR) receptors is necessary for effective resistance and transcriptional reprogramming of host cells. Co-IP and FRET-FLIM results show that EDS1 resides in nuclear complexes with several TNL receptors, suggesting that EDS1 is an immediate link between NLR-effector recognition and defense activation. Phylogenetic, molecular and protein structural data support actions of distinct EDS1-PAD4 and EDS1-SAG101 heterodimer complexes in TNL resistance. Although the N-terminal portions of EDS1 and PAD4 have the topology of a/b-hydrolase enzymes, they do not use an enzymatic mechanism for TNL or basal immunity. Instead, our data suggest that interaction between the a/b-hydrolase domains of EDS1 and its partners promotes alignment of the essential C-terminal a-helical bundle domains to form novel surfaces for signaling. We report on our structure-guided mutational analysis of the EDS1 signaling node and evidence for bacterial effector targeting of EDS1 as part of the resistance response.

Keywords: innate immunity, biotrophic pathogen, protein crystal structure, nuclear defense, transcriptional reprogramming

Poster #66. HSP90s are required for NLR immune receptor accumulation in Arabidopsis (Submission 60)
Shuai Huang1, Jacqueline Monaghan2, Xionghui Zhong3, Tongjun Sun1, Oliver Xiaou Dong1, Ling Lin2, Xin Li1
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Heat shock proteins (HSPs) serve as molecular chaperones for diverse client proteins in many biological processes. During the immune response, cytosolic HSP90s participate in immune receptor complex assembly, stability regulation and/or activation. In this study, we report that in addition to the well-established positive roles certain HSP90 isoforms play in plant immunity, HSP90s are also involved in the turnover of immune receptors. Point mutations in two HSP90 genes, HSP90.2 and HSP90.3, were identified from a forward-genetic screen designed to isolate mutants with enhanced disease resistance. We found that specific mutations in HSP90.2 and HSP90.3 lead to heightened accumulation of cytosolic immune receptors including SNC1, RPS2 and RPS4. We hypothesize that this occurs through HSP90-assisted formation of SCF E3 ubiquitin ligase complexes during immune receptor turnover or degradation. Such regulation is critical for maintaining appropriate immune receptor
protein levels to avoid autoimmunity.

Keywords: Plant Immunity, Resistance protein, HSP90, SCF, SNC1, RPS2, RPS4, Arabidopsis

Poster #67. Identification and characterization of an E3 ubiquitin ligase U4 in plant immunity (Submission 116)
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As a common post-translational modification, ubiquitination is used for regulating protein functions, notably in terms of stability. In recent years, ubiquitination has emerged as a major player in plant immunity, although not very many E3 ligases have been identified in this biological process, likely due to the high redundancies in E3 families in plants. In an effort to identify E3 ligases involved in plant defence, we selected 50 candidate E3-encoding genes whose transcripts are up-regulated upon plant defence, according to TAIR Microarray Expression databases. The chosen candidates were overexpressed in the autoimmune mutant snc1 background, and we screened for transgenic enhancers and suppressors that altered snc1-mediated autoimmunity. U4 is one of the suppressors, as its overexpression leads to compromised plant immunity and complete suppression of snc1-mediated autoimmunity. It is a predicted RING-type E3 ligase. An in vitro ubiquitination assay revealed that U4 exhibits E3 ligase activity, and overexpression of a dominant-negative version of U4 confers autoimmune phenotypes, confirming that U4 is indeed an E3 ligase. In summary, U4 plays an important role as a negative regulator in defense signaling, probably through targeting a key positive regulator for degradation. Future studies will be aimed at identifying the ubiquitination target of U4 to reveal how U4 regulates plant immunity.

Keywords: plant immunity, ubiquitination, E3 ubiquitin ligase

Poster #68. MUSE1 and MUSL redundantly regulate SNC1-mediated immunity (Submission 117)
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Negative regulation of plant immunity is critical for prevention of autoimmunity, which typically incurs a fitness cost. To search for negative regulators of plant immunity, we carried out a mutant, snc1-enhancing (muse) forward genetic screen. A number of novel genetic enhancers of the autoimmune mutant snc1 were isolated and characterized, including multiple alleles of muse1. Via positional cloning, MUSE1 was found to encode a RING domain protein. An in vitro ubiquitination assay demonstrated that MUSE1 possesses E3 ubiquitination ligase activity. BLAST analysis revealed that MUSE1 has a close paralog in Arabidopsis, MUSL. Although both muse1 and musl single mutants display wild-type-like morphology and disease resistance levels, the muse1 musl double knockout mutant exhibits extreme autoimmune phenotypes including dwarf stature with curly leaves, elevated expression of Pathogenesis-Related genes and enhanced disease resistance to the oomycete pathogen Hyaloperonospora arabidopsidis Noco2. Further epistasis analysis revealed that the autoimmune phenotypes of muse1 musl can be completely abolished by a loss-of-function allele of SNC1(snc1-r1), suggesting that MUSE1 and MUSL specifically and redundantly repress the defense signalling pathway mediated by SNC1. Future work will aim to identify the ubiquitination target(s) of MUSE1 to illustrate how MUSE1 regulates SNC1-mediated immunity.

Keywords: R protein-mediated defense, Ubiquitination E3 ligase, Autoimmunity

Poster #69. Pathogens in the Jam - Pectin and Plant Immunity (Submission 123)
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Plant cell walls are important early barriers preventing pathogen success. The cell walls of Arabidopsis thaliana
mesophyll cells consist mainly of cellulose, hemicelluloses and pectins. Pectins are synthesized in a highly methylesterified form in the Golgi and secreted into the apoplastic space. Pectin methylesterases (PMEs) de-esterify pectin in the plant cell wall. The methylesterification status of pectin is thought to influence mechanical properties of cell walls, since de-esterified pectin can bind calcium and form so-called "egg-box" structures. These are thought to increase the mechanical strength of pectin. Some plant pathogens such as Botrytis cinerea secrete PMEs that affect the virulence of these pathogens.

We have studied the role of PMEs in plant immunity. Inoculation of Arabidopsis leaves with Pseudomonas syringae pv. maculicola ES4326 (Pma ES4326) or Alternaria brassicicola induces plant-derived PME activity. This induction of PME activity is dependent on jasmonic acid (JA)-dependent signaling utilizing both the MYC2- and the ERF-dependent branches of JA signaling. Mutations in various PME genes cause enhanced susceptibility to Pma ES4326, but no difference in growth of Alternaria brassicicola can be detected. Interestingly, no differences in PME activity in these pme mutants could be observed, suggesting that specific PME genes have distinct functions and that the distribution of methylesters might be more important for immunity than total PME activity. UDP-D-glucuronate 4-epimerases (GAEs) are enzymes that convert UDP-D-glucuronate to UDP-D-galacturonate, the activated sugar precursor of pectin. Expression of GAEs is altered upon inoculation with various pathogens including B. cinerea and Pma ES4326. In the case of Pma ES4326 expression of GAE1 and GAE6 is repressed dependent on the master regulators of immunity, PAD4 and EDS1. Plants with mutations in GAEs are reduced in pectin and allow enhanced growth of Pma ES4326, suggesting that both pectin content and pattern of pectin modifications are important for plant immunity.

Keywords: plant-microbe interactions, plant cell wall, pectin

Poster #70. Identifying Arabidopsis thaliana genes for recognition of the HopAM1 type III effector from plant pathogenic Pseudomonas syringae. (Submission 128)
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Pseudomonas syringae is a gram-negative bacterium that causes disease on many plant species. The strain P. syringae DC3000, which can cause disease on Arabidopsis and Nicotiana benthamiana produces 30 effector proteins that are delivered through the Type Three Secretion System (TTSS) into the host cell to counteract plant defense responses. Our objective is to characterize the plant protein machinery that is affected by one of the type III effectors, HopAM1, which has a novel protein structure. We discovered natural variation between Arabidopsis ecotypes in their responses to 1) expression of HopAM1 in transgenic plants and 2) resistance responses to non-virulent Pseudomonads expressing HopAM1. Transgenic Arabidopsis of theWs-2 ecotype that express HopAM1 protein from an inducible promoter are inhibited in growth and show chlorosis in new leaves. This phenotype corresponds to reduced resistance to most strains of P. syringae bacteria (Goel et al., 2008). The ecotype Bur-0 has a semidominant suppressor allele that prevents the response to HopAM1 expression in F1 plants. We have mapped the suppressor locus to the top arm of Chromosome 2 using segregating F2 plants and fine mapping in progress. HopAM1 is also recognized by the ETI (effector triggered immunity) defense pathway (Jones and Dangl, 2006) in some ecotypes. When HopAM1 is delivered through non-pathogenic or "disarmed" bacteria, P. fluorescens (Thomas et al., 2009) or P. syringae DC3000D28E (Cunnac et al., 2011), it triggers a cell death response on Bur-0 ecotype plants but not on Col-0. Mapping of F2, backcross and RIL (Simon et al., 2008) populations derived from Bur-0 and Col-0 parents suggests that three major loci control HopAM1-dependent cell death. These loci are independent of alleles at the Chromosome 2 locus described above. We are characterizing segregating populations to identify the genes for the ETI resistance responses. By analysis of natural variation at multiple loci that affect responses to the bacterial virulence protein HopAM1, we hope to identify epistatic interactions that will improve our understanding of the plant immune system.

Keywords: Plant-pathogen interactions, Ecotype diversity, Segregation mapping

Poster/Talk #71. Acetylation of alternative N-terminal methionines oppositely controls the stability of a plant immune receptor (Submission 135)
In Arabidopsis, the callose synthase PMR4 (POWDERY MILDEW RESISTANT 4) is responsible for pathogen penetration. PMR4 overexpression in Arabidopsis resulted in an elevated early callose deposition at sites of attempted powdery mildew infection and complete penetration resistance. Despite the importance of PMR4-dependent callose biosynthesis in plant innate immunity and papilla formation, only little is known about its regulation and activation after infection. Results in yeast indicated an involvement of phosphorylation in the regulation of callose biosynthesis. Therefore, we mutated two PMR4 serine residues (S1, S2) to either alanine (A) to prevent phosphorylation or to aspartate (D) to mimic constant phosphorylation. Expression of the hydrophilic loop of PMR4 in Escherichia coli and subsequent purification facilitated an additional in vitro analysis of callose synthase activity. Almost complete loss of enzymatic activity of the S1-D1 mutated PMR4 peptide indicated an on/off function of the first serine residue, which was confirmed in PMR4 [S1-D1] overexpression lines. Moreover, the localization of PMR4 [S1-D1] at, but not the general transport to infection sites was altered compared to native PMR4 or PMR4 [S2-D2]. In contrast, the phosphorylation status of S2 is essential for PMR4 transport. S2-A2 mutation prevented transport of PMR4 to infection sites resulting in the absence localized callose deposition, which was on the other hand induced in S2-D2 mutated PMR4. Super-resolution microscopy additionally revealed differences in callose fibril orientation at infection sites, which were dependent on the S1 / S2 phosphorylation.
Poster #73. Pathogen-Induced Callose Deposition and Poly(ADP-ribosylation) (Submission 164)
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A well characterized chemical inhibitor of poly(ADP-ribosylation), 3-aminobenzamide (3AB), blocks specific plant responses to microbe-associated molecular patterns (MAMPs such as the flagellin epitope flg22), including callose cell wall reinforcement. Poly(ADP-ribosylation) is an important post-translational modification in which chains of poly(ADP-ribose) are added to a target protein. Poly(ADP-ribosylation) has been studied in mammalian systems for over 40 years and has received intensive focus due to its roles in DNA damage repair and cancer therapeutics. Much less is known about poly(ADP-ribosylation) in plants, but we have discovered roles in the plant innate immune response. A reverse genetics approach was utilized to examine the impact of parp1, parp2, and parp3 mutants as well as double and triple mutant combinations on MAMP-induced callose deposition. The parp1/parp2/parp3 triple mutants exhibit significantly reduced callose deposition compared to wild type, further implicating poly(ADP-ribosylation) in the plant innate immune response. However, the triple mutant does not exhibit a complete loss of callose deposition as is observed with 3AB treatment, opening the possibility that 3AB may have additional impacts beyond poly(ADP-ribosylation) to alter callose deposition. In order to identify additional targets of 3AB or downstream impacts, Arabidopsis natural variation is being utilized. Although 100uM 3AB effectively blocks callose deposition in many Arabidopsis accessions, others still produce substantial amounts of callose at this concentration. Efforts are currently underway to map the gene(s) underlying this variation in response to 3AB and could lead to the identification of novel targets of 3AB and previously unknown regulators of pathogen-induced callose deposition in Arabidopsis.

Keywords: plant-microbe interactions, innate immunity, callose deposition, cell wall reinforcement, poly(ADP-ribosylation)

Poster #74. Actin dynamics is a central node for innate immune signaling in plant cells (Submission 169)
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In both plants and animals, the recognition of microbe-associated molecular patterns (MAMPs) by pattern recognition receptors (PRRs) activates innate immune signaling, which further launches a cascade of defense responses including MAPK- and CDPK-dependent events. The actin cytoskeleton has been suggested as a central component for innate immune signaling and associated cellular responses. However, the molecular mechanisms that underpin actin remodeling and the precise functions of these rearrangements during innate immune signaling remain to be fully elucidated. Combining high spatiotemporal imaging and powerful tools for quantitative analysis of actin architecture and dynamics, we are able to test the rapid response of cytoskeletal remodeling to MAMP perception in plant epidermal cells. We demonstrate that the abundance of actin filaments increases within minutes in response to several distinct MAMP signaling pathways and that regulation of filament dynamics is a convergence point for basal defense machinery. Actin remodeling is necessary for cell wall fortification as well as transcriptional reprogramming through MAPK and CDPK signaling pathways. Our quantitative analyses of actin dynamics and genetic studies suggest that MAMP-stimulated actin remodeling is due to the inhibition of several key actin-binding proteins by cytoplasmic innate immune signals. In addition, we uncovered both parallel and convergent pathways for actin remodeling. Actin Depolymerizing Factor4 (ADF4) functions downstream of a signaling pathway elicited by the perception of a specific bacterial MAMP (elf26), whereas the ubiquitous barbed-end regulator, capping protein (CP), is identified as a universal target for multiple innate immune signaling events. Moreover, we demonstrate that the inhibition of CP by the signaling lipid,
phosphatidic acid, plays an essential role for actin remodeling in response to MAMP signaling. Collectively, our study provides deeper and broader understanding of the mechanisms underlying actin remodeling during plant innate immunity.

Keywords: Actin dynamics, innate immune signaling, plant-microbe interaction

Poster #75. Dissecting PAD4-independent immunity with chs3-2d pad4 (Submission 173)
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Most plant Resistance (R) proteins belong to the intracellular nucleotide-binding leucine-rich repeat (NB-LRR) protein family. TIR- and CC-NB-LRRs form the two major subgroups of NB-LRR proteins based on their N termini. EDS1 and PAD4, which are lipase-like proteins, are genetically required for many TIR-type R protein function. CHS3 (Chilling Sensitive 3) encodes an atypical TIR-NB-LRR protein with an additional zinc-binding LIM (Lin-11, Isl-1 and Mec-3) domain at its C terminus. A gain-of-function allele of CHS3, chs3-2d, shows constitutively activated defense responses including extremely dwarf stature, high PR gene expression and SA accumulation. Interestingly, the double mutant chs3-2d pad4 is slightly bigger than chs3-2d, while chs3-2d eds1 is wild type sized, indicating that chs3-2d-mediated autoimmunity is fully dependent on EDS1, but only partially dependent on PAD4. To search for novel regulators independent of PAD4, we conducted a suppressor screen in the background of chs3-2d pad4. Five second-site mutants that can either partially or completely suppress chs3-2d pad4 autoimmune phenotypes were isolated. Pair-wise allelism tests revealed that these five recessive mutants fall into four complementation groups. Crude mapping and Sanger sequencing of candidate genes revealed that two of them carry mutations in SGT1b, which encodes a TPR type co-chaperone previously known to be involved in CHS3-mediated immunity. Positional cloning is being attempted to identify two novel genes in this PAD4-independent pathway.

Keywords: Plant immunity, Resistance Protein, PAD4-independent, chs3-2d pad4

Poster #76. SARD1 and CBP60g function as master transcriptional regulators of early plant defense responses (Submission 179)
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Arabidopsis snc2-1D contains a gain-of-function mutation in a receptor-like protein resulting in constitutive activation of both salicylic acid (SA)-dependent immunity and SA-independent defense mediated by WRKY70. To test whether the increased synthesis of salicylic acid (SA) in snc2-1D relies on transcription factors SARD1 and CBP60g that are responsible for pathogen-induced SA synthesis, a sard1 cbp60g snc2-1D triple mutant was constructed. As expected, the SA level in the triple mutant is reduced to wild type level, suggesting that SARD1 and CBP60g regulate SA synthesis in snc2-1D. Surprisingly, SA-independent defense responses are also largely blocked in the triple mutant, indicating that SARD1 and CBP60g are also responsible for defense activation of the SA-independent pathway. Chromatin immunoprecipitation (ChIP)-sequencing analysis identified WRKY70 as a target gene of SARD1 and CBP60g. In the sard1 cbp60g snc2-1D triple mutant, expression level of WRKY70 is much lower than in snc2-1D, suggesting that SARD1 and CBP60g regulate SA-independent defense responses by controlling the expression of WRKY70. Further analysis identified many other known defense regulators as direct target genes of SARD1 and CBP60g. The expressions of these genes are regulated by SARD1 and CBP60g during early defense responses. In summary, our data suggest that SARD1 and CBP60g function as master regulators of plant defense during early transcriptional reprogramming.

Keywords: Plant immunity, Salicylic acid, SARD1, CBP60g, snc2-1D, WRKY70

Poster #77. The role of CDC48A in the negative regulation of R protein-mediated immunity (Submission 187)
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Keywords: Plant immunity, Resistance Protein, PAD4-independent, chs3-2d pad4
In order to be successful, all multicellular organisms must be able to defend themselves against potentially harmful microbes. Plant Resistance (R) proteins, many of which contain nucleotide-binding and leucine-rich repeat (NLR) domains, perceive effector proteins produced by invading pathogens and activate downstream defense signalling. This results in effective immunity against the pathogen. However, excessive accumulation of R proteins results in an autoimmune response even in the absence of pathogen interaction, which is detrimental to plant growth. Plants must therefore control R protein accumulation through delicate mechanisms, including degradation via the 26S proteasome pathway. However, the details of this regulation are still unclear. A forward genetic screen was conducted for negative regulators of R protein-mediated immunity, based on enhancement of the autoimmune phenotype of snc1, a gain-of-function NLR mutant. We identified a partial loss-of-function allele of CDC48A, which encodes an ATPase that is highly conserved throughout eukaryotes. Although CDC48A has an essential function in Arabidopsis, its role in plant immunity has not been reported. The cdc48A mutant displays a dwarf phenotype, constitutive Pathogenesis Related 2 gene expression, and enhanced disease resistance. The immune phenotype of cdc48A is dependent on EDS1 and is suppressed by high temperature, indicating that CDC48A may regulate Toll/Interleukin-1 Receptor (TIR) type R proteins. CDC48A interacts with MUSE3, an E4 enzyme known to target R proteins including SNC1 for degradation. Therefore, CDC48 may facilitate the proteasome-mediated degradation of R proteins such as SNC1.

Keywords: Immunity, R gene, Protein degradation

**Poster #78. Manipulation of ABA signalling by Pseudomonas syringae type III effectors** (Submission 188)
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The phytopathogen Pseudomonas syringae delivers type III effectors (T3Es) into host cells to increase virulence by suppressing host defence. One mechanism by which to accomplish this is to target hormone signalling pathways to perturb hormone homeostasis inside the host. Recent studies have elucidated the influence of the phytohormone abscisic acid (ABA) on the interaction between various phytopathogens and their plant hosts; including P. syringae and Arabidopsis. We hypothesize that P. syringae T3Es manipulate ABA signaling as a virulence strategy. To address this, we have generated a binary interaction map of P. syringae T3Es and Arabidopsis proteins that are transcriptionally regulated by ABA using high throughput yeast-two-hybrid analyses. As expected, the interactome contains Arabidopsis proteins of high interconnectivity (hubs), many of which are targeted by T3Es. Moreover, selected T3Es were identified that can affect the ABA interactome supporting our hypothesis that T3Es manipulate ABA signalling to promote P. syringae virulence.

Keywords: Pseudomonas syringae, abscisic acid, type III secreted effectors, biotic stress, plant immunity, phytohormones, interactome

**Poster #79. AtNUDX8 has a role in plant immunity through regulation of thioredoxins TRX-h2, TRX-h3 and TRX-h5 involved in pathogen response in Arabidopsis** (Submission 191)
Jose Pedro Fonseca¹, Xinnian Dong²
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Nudix hydrolases comprise a large gene family with twenty seven members in Arabidopsis. Until now only AtNUDX6 and ATNUDX7 genes were shown to be involved in defense responses. We showed in this study that a novel member of Nudix family, AtNUDX8, is involved in the plant defense machinery by modulating the expression levels of pathogen responsive thioredoxins TRX-h2, TRX-h3 and TRX-h5 which catalyze reduction of NPR1 protein disulphide bond formation and oligomer-monomer transition. AtNUDX8 displayed a typical circadian-like pulse expression with expression peaking at 8 hours and this expression was repressed four-fold by salicylic acid (SA). KO-nudx8 mutant showed enhanced disease susceptibility to bacterial pathogen Psm ES4326 and oomycete pathogen Hyaloperonospora arabidopsisdis compared to the wild-type control. KO-nudx8 mutant plants showed a small, stunted phenotype when grown in 12/12 hours photoperiod. Furthermore, KO-nudx8 plants like npr1 mutant, displayed SA hypersensitivity indicating that AtNUDX8 is involved in SA signaling. Indeed, basal expression of a key SA biosynthesis gene ICS1 was repressed in the KO-nudx8 suggesting that AtNUDX8
is involved in SA signaling in plants. Similarly, NPR1 transcript levels were also downregulated in the KO-nudx8 mutant indicating that AtNUDX8 gene is a positive regulator of NPR1 mediated defense in Arabidopsis. This is the first study on the functional characterization of AtNUDX8 in Arabidopsis. Our data indicates that AtNUDX8 is directly involved in plant immune responses by regulating pathogen responsive thioredoxins and that this regulation is under control of the circadian clock providing additional evidence to the link between the circadian clock and plant immunity in Arabidopsis.

**Keywords:** Arabidopsis, circadian, Nudix, NPR1, plant immunity, Thioredoxin

**Poster/Talk #80. Two redundant protein kinases act downstream of PAMP receptors to regulate activation of MAP kinases and SA synthesis in Arabidopsis** (Submission 193)

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Salicylic acid (SA) serves as a critical signal molecule in plant defense. Two transcription factors, SARD1 and CBP60g, control SA synthesis through regulating pathogen-induced expression of Isochrismate Synthase 1 (ICS1), which encodes a key enzyme for SA synthesis. Reverse genetic analysis of potentially redundant genes identified two proteins, Redundant Protein Kinase (RDPK) 1a and 1b, as positive regulators of SA synthesis. The rdpk1a rdpk1b double mutant, but not the single mutants, exhibited reduced accumulation of SA and enhanced disease susceptibility to virulent bacterial pathogens. Pathogen-induced expression of SARD1, CBP60g and ICS1 is also reduced in the double mutant. Further biochemical analysis revealed that RDPK1a interacts with PAMP receptor FLS2 and is rapidly phosphorylated in responses to flg22, elf18 and chitin. Activation of MAP kinases by flg22 and the non-pathogenic bacteria P.s.t. DC3000 hrcC is also attenuated in the rdpk1a rdpk1b double mutant. These data suggest that RDPK1a and RDPK1b function redundantly downstream of PAMP receptors in the signal relay leading to activation of MAP kinases and SA synthesis.

**Keywords:** Salicylic acid, SARD1, CBP60g, MAP kinases

**Poster/Talk #81. Salicylic acid mediated defence is fine-tuned through the interplay of calmodulin-binding proteins and calmodulin-like proteins in Arabidopsis** (Submission 208)

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Salicylic acid (SA) is a key signalling compound for defence responses in Arabidopsis. Three members of the CBP60 gene family have been characterised as playing differential roles in regulating immune responses. CBP60g and SARD1 are positive regulators of defence with mutations conferring increased susceptibility to the bacterial pathogen Pseudomonas syringae pv maculicola ES4326 (Pma). Conversely, cbp60a mutants are more resistant to Pma infection. CBP60g and SARD1 have been shown to regulate expression of the key SA biosynthesis gene SID2, binding to GAAATTT motifs in the SID2 promoter. CBP60g and SARD1 are required for the full induction of SID2 expression and SA accumulation in response to Pma infection; cbp60a mutants exhibit partial activation of these defences in the absence of infection. Calmodulin binding ability is required for the positive regulatory role of CBP60g and the negative regulatory role of CBP60a. Two members of the calmodulin-like gene family, CML46 and CML47, are tightly co-expressed with CBP60g, SARD1 and SID2 in a cluster of genes with GAAATTT motif enriched promoters. The Pma induced expression of CML46 and CML47 is affected by both CBP60g/SARD1 and CBP60a. Loss of either CML46 or CML47 results in restriction of Pma growth while this effect is increased in the cml46 cml47 double mutant. Like cbp60a, uninfected cml46 mutants have elevated SA levels indicating they may also play a role in suppressing accidental triggering of costly SA mediated defences.

**Keywords:** plant-microbe interactions, calcium signalling, Pseudomonas syringae, calmodulin, salicylic acid, plant immunity

**Poster/Talk #82. Using plant pathogen effectors as evolved probes to understand the plant immune system** (Submission 209)
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The plant innate immune system contains a limited number of receptors. Exactly how this limited set of immune receptors functions to identify all potential pathogens is still largely not understood. Intracellular pathogen detection is performed by nucleotide-binding oligomerization domain-like receptor (NLR) proteins, which are defined by their stereotypical multidomain structure: an N-terminal Toll-interleukin receptor (TIR) or coiled-coil domain, a central nucleotide-binding (NB) domain and a C-terminal leucine-rich repeat (LRR) domain. Using a genetic approach exploiting natural variation, we have isolated a novel resistance gene (response to hopBA1, RBA1) that lacks most of the characteristic domains thought to be required for NLR function. This gene demonstrates a function for previously uncharacterized "truncated" NLRs and prompts us to reconsider the current understanding of NLR receptor domain structure.

To identify novel components of the plant immune system we assayed natural variation of Arabidopsis in response to bacterial virulence factors. One important and large class of virulence factors from plant pathogenic bacteria is the type III effector (TTE) proteins. These proteins are injected directly into the plant cytoplasm, where they interact with a set of host targets to promote virulence. By identifying, cloning and screening ~90 effectors on a set of ~90 Arabidopsis accessions we have identified interactions that display genotypic diversity. We have determined the structure of one of the novel effectors, HopBA1, and found that is recognized by several accessions, resulting in cell death. By positionally cloning the Arabidopsis locus (RBA1) responsible for recognition of hopBA1 we have identified the first member of an unusual class of TIR-domain NLR-like genes. This class of "truncated" receptors lacks the NB and LRR domains that are characteristic of known plant NLR proteins. We hope that the hopBA1/RBA1 system will provide broad insights into the diversity and function of innate immune proteins by revealing previously undescribed modularity that could account for an increased diversity of recognition.

Keywords: Plant-pathogen interactions, Plant immune system, Natural variation, NLR protein, TIR domain, Type III effector

**Poster #83. Cellulose-derived oligomers activate defense responses in Arabidopsis thaliana.** (Submission 210)
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During a typical fungal infection, pathogens secrete hydrolase enzymes that target the plant cell wall, generating Damage Associated Molecular Patterns (DAMPs). In addition to recognizing a pathogen presence through perception of Pathogen Associate Molecular Patterns (PAMPs), plants perceive DAMP fragments and respond by activation of the first line of defense, termed PTI (Pathogen Triggered Immunity). PTI processes include intracellular calcium spike, generation of Reactive Oxygen Species (ROS), protein phosphorylation, and differential gene expression. Oligomers of pectin (ie. Oligogalacturonans, or OGs) are well characterized DAMPs that are perceived by Wall Associate Kinase 1 (WAK1). Our lab is interested in identifying new DAMPs and understanding the role of DAMP perception in the overall defense response in Arabidopsis. We conducted a screen for cell wall-derived fragments that lead to a defense response in Arabidopsis, and characterized in detail the response triggered by application of exogenous cellulose-derived oligomers, such as cellobiose. Our results indicate that cellobiose is perceived as a DAMP, generating a rapid calcium spike and activation of Mitogen-Activated Protein Kinase 6 (MAPK6), a well characterized component of PTI signaling. Furthermore, analysis of gene expression following cellobiose treatment indicates the response is similar to that of chitin and OGs, however, in contrast to other PAMPs, cellobiose treatment does not generate detectable ROS. By treating Arabidopsis seedlings with combinations of elicitors we observe an amplification of the PTI response. We are currently exploring the hypothesis that the detection of PAMP+DAMP combination may confer higher specificity and help prevent unnecessary activation of PTI. Additionally, we are working to identify components of cellobiose perception and response via a genetic screen.

Keywords: PTI, cell wall, cellobiose, PAMP/DAMP perception, defense signaling
Poster/Talk #84. Decreased abundance of type III-inducing signals in Arabidopsis mkp1 enhances resistance to Pseudomonas syringae (Submission 214)
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The type III secretion system (T3SS) is a virulence mechanism used by the bacterial pathogen Pseudomonas syringae to deliver an array of effector proteins into plant cells. Once inside, these effectors suppress host defense responses, thereby allowing the bacteria to escape detection and proliferate. Although effector-mediated defense suppression appears to be a critical component of P. syringae virulence (e.g. T3SS-deficient mutants are non-pathogenic), the T3SS is not constitutively present in the bacteria and must be produced during the initial stages of infection. Various plant-derived signals that induce T3SS-encoding genes in P. syringae have been proposed based largely on in vitro experiments. However, whether any of these signals affect the outcome of P. syringae-host interactions is unclear because of the lack of genetic mutants that alter their abundance in the host.

Here we report that type III effector delivery by P. syringae pv tomato DC3000 is reduced in mkp1 (mapk phosphatase 1), an Arabidopsis mutant that is more resistant to bacterial infection [1]. To investigate the mechanism(s) responsible for decreased effector delivery, we performed a metabolomic comparison of exudates prepared from wild type and mkp1 plants and discovered that several T3SS-inducing metabolites were present at decreased levels in mkp1 [2]. We next reintroduced the T3SS-inducing metabolites during DC3000 infection of Arabidopsis and found that both the decreased effector delivery and enhanced resistance observed in mkp1 plants were completely suppressed. These results indicate that the mechanism of resistance against DC3000 infection in mkp1 is a lack of chemical cues that initiate bacterial virulence. Pretreatment of plants with pathogen associated molecular patterns (PAMPs) to induce PAMP-triggered immunity (PTI) also restricts T3SS effector delivery and enhances resistance to bacterial infection by unknown mechanisms. Similar to results with mkp1, addition of the bioactive metabolites suppressed both aspects of PTI in Arabidopsis against DC3000 infection. Together, these results demonstrate that DC3000 perceives multiple signals derived from plants to initiate its T3SS and that the level of these host-derived signals impacts bacterial pathogenesis.

References

Keywords: Pseudomonas syringae, type III secretion system, metabolomics, PAMP-triggered immunity, type III effectors

Poster #85. Functional Diversification of the HopF Type III Secreted Effector Family (Submission 215)
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Equipped with an arsenal of virulence proteins, termed type III secreted effectors (T3SEs), the Gram-negative bacterial phytopathogen Pseudomonas syringae can infect a wide range of plant hosts, including Arabidopsis. Numerous families of T3SEs exist to promote P. syringae infection through suppression of plant immunity, including the HopF family. Although research on this T3SE family has focused on two members, the HopF2 from P. syringae pathovar tomato DC3000 (PtoDC3000) and HopF1 from P. syringae pathovar phaseolicola 1449B (Pph1449B), the importance of other family members have yet to be investigated. With the increasing number of sequenced P. syringae genomes, the HopF family has greatly expanded to over forty different members, which can be divided into six different subgroups including two groups of chimeric T3SEs only partially homologous to the rest of the HopF family. This study aims to explore the roles of the uncharacterized HopF family members and to examine the function of the HopF family as a whole. Functional characterization was undertaken in Arabidopsis to explore the functional diversification of the HopF family of T3SEs. We have identified a novel immune response in Arabidopsis triggered by the T3SE HopF2 from P. syringae pathovar tomato T1 and we will present the genetic characterization of this response.

Keywords: Pseudomonas syringae, Type III secreted effectors, Plant pathology, Effector triggered Immunity, ETI, Evolutionary arms race, Plant immunity
Poster/Talk #86. Pseudomonas syringae effector AvrE is a novel phosphatidylinositol-binding protein that down-regulates the expression of NDR1/HIN1-1-Like 13 required for immunity (Submission 226)
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The AvrE family of type III effectors is among the most crucial virulence factors in Gram-negative phytopathogenic bacteria that cause a variety of diseases in plants. Despite two decades of studies, the molecular action of AvrE-family effectors remains enigmatic, leaving a significant gap in our understanding of bacterial diseases in plants. In this study, a serial deletion analysis revealed that the N- and C-terminal halves of the 1,795-aa AvrE protein from Pseudomonas syringae pv. tomato (Pst) DC3000 represent two interacting modular domains. Genome-wide gene expression analysis revealed that the virulence function of AvrE during Pst DC3000 infection leads to down-regulation of NHL13, a member of the defense-associated NDR1/HIN1-like (NHL) family. Importantly, Arabidopsis nhl13 knock-out mutant plants are compromised in immunity. AvrE is localized in the plasma membrane (PM) in plant cells, with the PM-targeting signal residing within the N-terminal half. Through bioinformatic and biochemical analyses, we found that the N-terminal half of AvrE contains a [?] -propeller that binds phosphatidylinositides (PIPs), a property shared by eukaryotic PROPPIN-family proteins. Two highly conserved GxxY motifs in the [?] -propeller domain of AvrE were found to be critical for AvrE's virulence activity in the host cell. Thus, we have identified AvrE as a novel type of bacterial PIP-binding protein that shares features of eukaryotic PROPPINs. Moreover, our results show that the Arabidopsis NHL13 gene as a component of the plant immune system and a downstream transcriptional target of AvrE's virulence function.

Keywords: type III effector, plant immunity, bacterial virulence, phosphatidylinositol-binding, plant pathogen

Poster #87. Towards elucidation of the jasmonate-independent function of the jasmonate receptor COI1 in mediating susceptibility against the vascular pathogen Verticillium longisporum (Submission 250)
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Verticillium longisporum is a soil-borne vascular pathogen that causes major yield losses on rapeseed (Brassica napus). Our previous analysis of the V. longisporum/Arabidopsis thaliana interaction has unraveled two unexpected findings: (1) COI1, the receptor of the plant defense hormone jasmonic acid-isoleucine (JA-Ile), is required in roots for efficient proliferation of the fungus in the shoot. (2) The novel COI1 function operates even in the absence of plant-derived JA-Ile or fungus-derived JA-Ile mimics.
The long-term goals of our research are to elucidate root-borne COI1-dependent processes that are independent of JA-Ile. Next generation sequencing of RNA from wild-type and mutant roots has identified a number of genes that are expressed to higher levels in the coi1 mutant as compared to wild-type and the JA biosynthesis mutant aos. Elevated expression of these genes in coi1 can be suppressed again by ectopic expression of mutated COI1 proteins that are not able to bind JA-Ile due to site specific mutations in the JA-Ile binding pocket. Since COI1, but not JA-Ile, can be detected in the moss Physcomitrella patens and since a target gene of this JA-Ile-independent COI1 function affects an enzyme of glycolysis, the novel role of COI1 as a JA-Ile-independent repressor of gene expression might represent an evolutionary ancient function of the protein.

Keywords: susceptibility, COI1, jasmonic acid, root-borne signal, vascular pathogen

Poster #88. Splicing of Receptor-like kinase-encoding SNC4 and CERK1 is regulated by two conserved splicing factors that are required for plant immunity (Submission 291)
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Plant immune receptors belonging to the receptor-like kinase (RLK) family play important roles in the recognition of microbial pathogens and activation of downstream defense responses. The Arabidopsis mutant snc4-1D contains a gain-of-function mutation in the RLK SNC4 (SUPPRESSOR OF NPR1-1, CONSTITUTIVE4), which leads to constitutive activation of defense responses. Analysis of suppressor mutants of snc4-1D identified two
conserved splicing factors, RSN1 (REQUIRED FOR SNC4-1D 1) and RSN2, that are required for the constitutive defense responses in snc4-1D. In rsn1 and rsn2 mutants, SNC4 splicing is altered and the amount of SNC4 transcripts is reduced. Further analysis showed that RSN1 and RSN2 are also required for the proper splicing of CERK1 (CHITIN ELICITOR RECEPTOR KINASE1), which encodes another RLK that functions as a receptor for chitin. In rsn1 and rsn2 mutants, induction of reactive oxygen species by chitin is reduced and the non-pathogenic bacteria P.s.t. DC3000 hrcC grows to higher titers than in wild type plants. Our study suggests that pre-mRNA splicing plays important roles in the regulation of plant immunity mediated by the RLKs SNC4 and CERK1.

Keywords: Plant immunity, Splicing factors, Receptor like kinases

Poster #89. Identification of plant non-retroviral-like sequences present in Arabidopsis genome (Submission 295)
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Viruses are infectious agents with relatively small genome sizes and rely on their life cycles within the living host cells. With rapid recent advances in genome data for viruses and their hosts, it is now possible to trace the origins and evolution of viruses along with their hosts. In order to identify non-retroviral-like sequences present in Arabidopsis genome as a model plant, we intensively conducted BLAST search against Arabidopsis genome using available plant non-retroviral sequences. As a result, we revealed 156 viral sequences which were homologous to 51 Arabidopsis proteins. Identified viral-like sequences were derived from four major virus groups including single-stranded (ss) RNA viruses (84%), double-stranded (ds) DNA viruses (7%), dsRNA viruses (5%), and other viruses (4%). The identified most viruses are members of the family Potyviridae (54%), followed by the family Closteroviridae (22%) and the family Caulimoviridae (7%). The significantly enriched plant viruses are members of the genus Potyvirus (44%) followed by the genus Closterovirus (8%), the genus Crinivirus (8%), the genus Badnavirus (6%), and the genus Ampelovirus (4%). The heat shock protein (31 viruses) and cylindrical inclusion protein (30 viruses) are most prominent viral proteins, which are homologous to Arabidopsis proteins. Capsid protein (12 viruses), RNA dependent RNA polymerase (6 viruses), helicase (3 viruses), RNaseH (2 viruses) were also frequently identified proteins. To reveal putative functional roles of viral-like sequences in Arabidopsis, we carried out gene ontology analysis. Most genes are involved in stress response and various metabolic processes. In addition, we performed BLAST search against available 41 plant genomes. Interestingly, we revealed that integration of viral genes was dependent on different plant species. Taken together, this study is a genome-wide identification of plant non-retroviral-like sequences present in Arabidopsis genome to study evolutionary origins of plant viruses and to explain contribution of integrated viral sequences to plant genomes during plant-virus co-evolution.

Keywords: Virus, Arabidopsis, Plant-virus co-evolution

Poster #90. Effect of bacterial lipopolysaccharides on the microtranscriptome of Arabidopsis thaliana leaf and callus tissues (Submission 311)
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MicroRNAs (miRNAs) are non-coding RNA molecules which have recently emerged as important gene regulators in plants and their gene expression analysis is becoming increasingly important. miRNAs regulate gene expression at the post-transcriptional level by translational repression or target degradation of specific mRNAs and gene silencing. In order to profile the microtranscriptome of Arabidopsis thaliana leaf and callus tissues in response to bacterial lipopolysaccharides, small RNA libraries were constructed at 0 and 3 h post infection and sequenced by the Illumina sequencing method. After sequencing and subsequent analyses we mapped small RNA reads to the microRNA database (miRBase 20.0) and identified in the callus tissues 358 miRNAs belonging to 49 arabidopsis miRNA families and in the leaf tissues 272 miRNAs belonging to 40 arabidopsis miRNA families. Moreover, target genes for all the identified miRNAs families in the leaves were found but in the case of callus tissue, target genes for 44 of the 49 miRNAs families were found. In both leaf and callus, in response to elicitation by bacterial lipopolysaccharides, the majority of miRNAs were differentially expressed. The expression
profile of miR156, miR169, miR398 and miR408 measured by the quantitative real-time PCR validated the sequencing results. Our study provided valuable information to understand the function of miRNAs in the regulation of the plant response to biotic stress and to add insight into the action mechanism of LPS.

Keywords: Arabidopsis thaliana, Bacterial lipopolysaccharides, MicroRNAs, Illumina sequencing, Expression profiles

**Poster #91. Genetic analysis of receptor-like protein SNC2-mediated plant resistance in Arabidopsis** (Submission 341)
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Plants can detect microbial pathogens via the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). A small number of these receptors belong to the receptor-like protein family. Previously, we showed that a gain-of-function mutation in Arabidopsis receptor-like protein SNC2 (Suppressor of NPR1, Constitutive2) leads to constitutive activation of defense responses in snc2-1D mutant plants. To identify new components involved in SNC2-mediated resistance pathway, we carried out a suppressor screen in the eds5-3 snc2-1D npr1-1 mutant background. Map-based cloning of one of the suppressor genes, BDA3 (for bian da; "becoming big" in Chinese), showed that it encodes a RING (Really Interesting New Gene) domain containing protein. The mutation in BDA3 suppresses the dwarf morphology and constitutive defense responses in eds5-3 snc2-1D npr1-1 and results in enhanced susceptibility to oomycete pathogen Hyaloperonospora arabidopsidis (H.a.) Noco2. In addition, several loss-of-function alleles of FMO1 and ALD1 were also identified as bda mutants in the suppressor screen. Future work will focus on functional analysis of BDA3 protein and the roles of FMO1 and ALD1 in SNC2-mediated resistance pathway.

Keywords: plant immunity, SNC2, BDAs, FMO1, ALD1

**Poster #92. Identification of mitogen-activated protein kinases involved in pathogen-associated molecular pattern (PAMP)-triggered immunity** (Submission 345)
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Mitogen-activated protein (MAP) kinase cascades consist of MAP kinase kinase kinases, MAP kinase kinases and MAP kinases which are sequentially phosphorylated. Phosphorylation of MAP kinases by the upstream MAP kinase kinase leads to activation of the MAP kinases. Phosphorylated MAP kinases can be detected by the a-phospho-p44/42-ERK, which is specific for phosphorylated MAP kinases. In Arabidopsis, three immunoreactive bands are detected upon PAMP treatment using a-phospho-p44/42-ERK. Previous studies showed that the upper two bands are phosphorylated MPK6 and MPK3, respectively. The lower band consists of phosphorylated MPK4 and MPK11 as well as MPKs with unknown identity. To identify the additional MAP kinases phosphorylated upon PAMP perception, seven candidate MPKs with similar sizes as MPK4 were selected for further analysis. Transgenic plants expressing these MPKs with a ZZ-3xFLAG double tag were generated in Col-0 background. The double tag increases the size of the fusion protein by 17 kD, allowing for the detection of the phosphorylation of individual MAP kinases apart from the endogenous proteins. Western blot analysis showed that MPK1, MPK2, MPK7, MPK11 and MPK13 were phosphorylated upon PAMP treatment. Bacterial growth tests showed no significant difference between Col-0 and the single mpk mutants, suggesting potential functional redundancy between these MPKs. Future studies will focus on the characterization of the roles of these MAP kinases in plant immunity.

Keywords: Plant immunity, MAP kinase, PAMP-triggered immunity

**Poster #93. The viral protein P19 requires host factors for successful suppression of RNA silencing in Arabidopsis thaliana.** (Submission 374)
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**Poster #94. Regulation of SOBIR1 accumulation and activation of defense responses in bir1-1 by specific components of ER quality control** (Submission 391)

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Arabidopsis BAK1-INTERACTING RECEPTOR-LIKE KINASE 1 (BIR1) functions as a negative regulator of plant immunity. bir1-1 mutant plants display spontaneous cell death and constitutive defense responses that are dependent on SUPPRESSOR OF BIR1, 1 (SOBIR1) and PHYTOALEXIN DEFICIENT4 (PAD4). We found that mutations in four components of ER quality control, CALRETICULIN3 (CRT3), ER-LOCALIZED DnaJ-LIKE PROTEIN 3b (ERdj3b) STROMAL-DERIVED FACTOR-2 (SDF2) and UDP-glucose: glycoprotein glucosyltransferase (UGGT) can suppress the spontaneous cell death and constitutive defense responses in bir1-1. Further analysis revealed that accumulation of the SOBIR1 protein is reduced in crt3-1 and erdj3b-1 mutant plants. Our study suggests that ER quality control plays important roles in the biogenesis of SOBIR1, and is required for cell death and defense responses in bir1-1.

Keywords: plant immunity, ER-quality control, SOBIR1, CRT3, Erdj3b, SDF2, UGGT

**Poster #95. Phosphorylation is a Key Mechanism in stress-induced Callose Biosynthesis** (Submission 399)

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The deposition of the (1,3)-β-glucan callose in papillae, cell wall thickenings at sites of attempted pathogen penetration, is an early plant defense response to prevent or slow pathogen ingress. In Arabidopsis thaliana, the callose synthase PMR4 (POWDERY MILDEW RESISTANT 4) is responsible for callose biosynthesis after stress. Despite the importance of PMR4 in early defense response, only little is known about its regulation. Based on results in yeast that indicated the involvement of phosphorylation in regulating callose biosynthesis, we mutated a serine (S), which was identified to be phosphorylated to an alanine (A) or aspartate (D) to prevent or mimic phosphorylation at this residue of PMR4, respectively. In contrast to the callose synthase disruption line pmr4 without stress-induced callose deposition, overexpression of the native and S→A mutated PMR4 in the genetic background of pmr4 resulted in callose deposition at infection sites 6 hours post-inoculation (hpi) with the powdery mildew Golovinomyces cichoracearum. Reassembling the pmr4 disruption mutant, callose deposition was almost absent in the PMR4 overexpression line with the S→D deposition 6 hpi. Also 24 h post-inoculation, only slight callose deposition was observed at penetration sites in the S→D mutant in contrast to the strong callose accumulation in the S→A mutant and native PMR4 overexpression lines. At 7days
post-inoculation, the number of conidiophores on infected leaves in S->D lines was similar to that observed for the pmr4 disruption mutant whereas number of conidiophores was higher on infected leaves of the S->A lines.
To test whether the observed phenotypes for callose depositions would be associated with the phosphorylation status of PMR4, we expressed the intracellular loop of native, S->A- and S->D-mutated PMR4, which contains the putative catalytic domain of this callose synthase, in Escherichia coli. After purification of the intracellular loop via an attached Strep-tag, we determined callose synthase activity in an ELISA-based assay. Our data of in vitro callose synthase activity revealed that S->A mutation of PMR4 results in a similar callose production like native PMR4 whereas catalytic activity of S->D-mutated PMR4 was strongly reduced, explaining the observed callose deposition after powdery mildew infection.
The results suggest that dephosphorylation at the examined serine residue is required for full callose synthase activity of PMR4 after biotic stress.

Keywords: Arabidopsis thaliana, Golovinomyces cichoracearum, Callose deposition, PMR4, Phosphorylation, in vitro activity assays

Poster #96. Involvement of CLE peptide signaling in nematode infection process (Submission 402)
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Root-knot nematodes are obligate plant parasites that are distributed worldwide, and they cause serious damage to crops. During infection, nematodes recognize root tips, and penetrate the roots directly. After invasion, they induce galls by the injection of various effectors that are thought to suppress disease resistance mechanisms of host plant and trigger trans-differentiation. The plant cells are trans-differentiated into multinucleated giant cells, which are source of nutrition for nematodes. We have found several candidates of the effector protein from salivary gland of nematodes (Meloidogyne hapla) using matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS). Currently, we are analyzing biological relevance of the candidates. In addition to the effector proteins, CLAVATA3/EMBRYO SURROUNDING REGION (CLE)-like peptides encoded on the nematode’s genome are predicted as effector. In plant, CLE peptides are known to be involved in control of meristem activity through leucine-rich repeat receptor-like kinase (LRR-RLK) pathway, e.g. CLAVATA1 (CLV1), CLV2, BARELY ANY MERISTEM1 (BAM1). Nematode CLE-like peptides are highly resemble to plant CLE peptide, and showed similar effect of plant CLE peptides. CLE genes are conserved in many plants, in contrast, only phytoparasitic nematodes have CLE genes in animal kingdom. In this study, we analysed the infection process of root-knot nematode (Meloidogyne incognita) against Arabidopsis as a model system, to elucidate the molecular mechanism. We show that clv2 and clv1 mutants resist M. incognita CLE-like peptides treatment and clv1 mutants showed resistance to the nematode infection. In contrast, bam1 and clv3 mutants are susceptible to nematode infection. Additionally, our expression analyses revealed that various CLV pathway related genes are affected during gall development. These results suggest that CLV signaling pathway is involved in nematode infection process.

Keywords: Root-knot nematode, Giant cells, Pathogenicity, CLE peptide

Poster #97. Requirement of the chloroplast DNA repair protein RECA1 for the immune response in Arabidopsis (Submission 411)
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Chloroplast, the descendant of a cyanobacterial ancestor that established symbiotic relationship with ancient eukaryotic host, is involved in various biological processes including biotic stress response in higher plants. It was investigated whether RECA1, a chloroplast homolog of the bacterial DNA recombinase RecA, might be involved in biotic stress response in Arabidopsis. First, the RECA1 transcripts were found to be induced upon treatments of the plants with BTH, SA, MeJA, ethephon, and flg22, respectively. Microarray experiments showed that RECA1 overexpression in Arabidopsis increased the expression of numerous genes, including the defense genes that accounted for about 20% of the up-regulated ones by RECA1. These defense genes covered a broad range of plant defense signaling against bacterial pathogen, encoding the upstream PAMP- or effector-recognizing
receptors, the intermediate defense signaling components, and the downstream PR proteins. RECA1-overexpressing transgenic plants showed higher levels of SA accumulation than wild-type, whilst recA1 mutant showed the opposite results. Consistently, the genes involved in SA biosynthesis were up-regulated by RECA1. All of these results were in harmony with the resistance of RECA1-overexpressing plants or the susceptibility of recA1 mutant to Pst DC3000. Genetic experiments further showed that RECA1 plays an important role in the NPR1-dependent, SA-induced activation of PR gene expressions. Combined together, it was concluded that the chloroplast RECA1 is required for the immune response of higher plants, which is thought to have been developed via inter-organellar signaling over the course of plant evolution.

Keywords: chloroplast DNA repair protein, plant immune response, inter-organellar signaling

**Poster #98. Arabidopsis Immunity Triggered by the HopF Family of Type III Effectors (Submission 430)**

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Pseudomonas syringae is a Gram-negative bacterial phytopathogen that causes disease in a broad range of plant species. An essential virulence strategy of P. syringae involves injecting type III secretion effectors (TTSEs) into host cells to inhibit immune signaling. However, plants actively monitor for the presence of TTSEs using resistance proteins (R proteins), which trigger an immune response referred to as effector-triggered immunity (ETI). The Arabidopsis protein RIN4 is targeted by three unrelated TTSEs AvrRpm1, AvrB, and AvrRpt2 which trigger ETI via the R proteins RPM1 and RPS2 to effectively restrict pathogen growth. However, another TTSE, HopF2PtoDC3000 targets RIN4 to promote P. syringae growth. Currently, it is unclear how some TTSE target RIN4 to trigger immunity while others can target RIN4 to disable immunity. The HopF family of TTSEs contains over 20 diverse family members, however, research thus far has focused on two family members. Our expanded functional analysis of the HopF family has revealed members that can trigger ETI in Arabidopsis. We will present our genetic analysis of this ETI response.

Keywords: pseudomonas syringae, Effector triggered immunity, Arabidopsis, AvrRpm1, RIN4, HopF

**Poster #99. Folylpolyglutamate Synthase and Host-Pathogen Interactions (Submission 431)**

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In recent years, poly(ADP-ribosyl)ation has been implicated in the regulation of a variety of plant defense responses to both abiotic and biotic stressors. The exact mechanism by which this post-translational modification regulates plant stress responses has yet to be elucidated. Previously, we identified Fpgs3 (Folylpolyglutamate synthase 3) (At3g55630) in a transcriptomics study of those Arabidopsis thaliana basal immune response regulatory genes disrupted by genetic knockout of a poly(ADP-ribose) glycohydrolase (Parg1) gene. Folylpolyglutamate synthases catalyze the polyglutamylation of folate, and this one-carbon metabolic reaction is necessary for purine biosynthesis (including adenylates such as ADP-ribose) and folate homeostasis. The goal of the present study was to investigate the role of FPGS enzymes in the plant innate immune response and poly(ADP-ribosyl)ation reactions. There are at least four genes in the Arabidopsis genome predicted to encode FPGS-type enzymes. Multiple T-DNA insertion knockouts of each of these genes were acquired and tested for biotic stress phenotypes. fpgs2 (At3g10160) knockout plants display reduced callose deposition and attenuated seedling growth inhibition in response to the MAMPs (microbe-associated molecular patterns) flg22 and elf18. Further molecular and phenotypic characterization of single and double Fpgs knockouts will be presented. It has been hypothesized that the consumption of NAD+ by poly(ADP-ribose) polymerases disrupts the energy and redox homeostasis in plant cells, causing the observed disruptions in abiotic and biotic stress responses. Folate polyglutamylation may provide one mechanism by which cells protect themselves from such stress-induced energy homeostasis disruptions.

Keywords: MAMPs, Defense response, ADP-ribose, Pseudomonas syringae
Poster #100. The role of chromatin modifications on molecular priming of the plant immune system (Submission 443)
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Priming is the indirect enhancement of the immune response of plants to pathogens. Compared to unprimed plants, the immune response from primed plants upon pathogen attack is much stronger. My research project focuses on the priming effects of plant defense responses against pathogenic attack. Recent research in Arabidopsis thaliana has shown that the plant hormone cytokinin, along with many other organic and inorganic chemicals, has a priming effect on plants. Even though the priming phenomenon has been observed, the molecular mechanisms behind it remain unknown. Many studies recently proposed that chromatin modifications, such as methylation/de-methylation and acetylation/de-acetylation, may play a role in priming. We identified genes encoding transcription factors and DNA binding proteins whose expression is regulated by cytokinin. One of these genes, HISTONE DEACETYLTRANSFERASE 3 (HDT3), encodes a DNA binding protein with histone deacetylation activity, and therefore is believed to work as a negative regulator of gene expression. Because of the similarities on how known priming agents and cytokinins potentiate defense gene expression, it is possible to hypothesize that priming in Arabidopsis by cytokinin may also involve chromatin modifications, possibly mediated by the HDT3 gene. My project involves testing the role of HDT3 on priming of the plant immune system, through the use gene expression and genetic analyses.

Keywords: Defense Priming, Cytokinin, Histone Deacetyltransferase, Biotic Interactions, Chromatin modification

Poster #101. Use of enhancer trapping to identify pathogen-induced regulatory events spatially restricted to plant-microbe interaction sites (Submission 452)
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The plant immune system involves intricate genetically regulated responses to pathogens. Plant genes differentially expressed during plant/pathogen interactions can be important for host immunity or contribute to pathogen virulence. Microarray analyses and other large scale transcript profiling studies have revealed hundreds of genes that are active during plant pathogen interactions. However, transcriptional responses limited to a small number of cells at infection sites can be difficult to detect by such methods as they are under-represented in whole tissue data sets. This study examined interactions between Arabidopsis thaliana (Arabidopsis) and the pathogenic oomycete Hyaloperonospora arabidopsidis (Hpa) and used Arabidopsis enhancer-trap lines to uncover novel plant genes involved in local infection responses. Enhancer trapping is an effective method for the identification of gene expression patterns of interest. We screened the Thomas Jack β-glucuronidase (GUS) reporter-based enhancer-trap population¹, comprised of over 11,000 independent insertion lines, for expression patterns related to Hpa infection. Seven independent lines exhibited GUS expression in leaf mesophyll cells surrounding Hpa structures, indicating a response to pathogen infection. One of these lines contained a single enhancer-trap insertion in an exon of At1g08800 (MYOB1) and was found to exhibit reduced susceptibility to Hpa. Two additional plant lines with T-DNA insertions in exons of MYOB1 also showed approximately 30% fewer spores than wild type plants. Thus, MYOB1 either positively contributes to Hpa virulence or negatively affects host immunity against this pathogen. Transgenic lines with a construct containing the entire MYOB1 promoter region fused to the GUS reporter gene mimic responses by the original enhancer-trap line when exposed to various defense-related compounds and treatments. This verifies the reporter gene expression pattern initially seen in the screen and its association with the MYOB1 promoter region. Such spatially-restricted plant defense responses can provide key information about locally controlled, highly specific defense mechanisms. This research was funded by USDA grant 2008-35301-19264.


Keywords: plant defense, enhancer trapping, Hyaloperonospora arabidopsidis
Poster #102. Plant immunity control by MAPK phosphatases (Submission 461)
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Environmental signals are perceived and transmitted in cells to induce appropriate responses. Bacterial pathogens activate mitogen-activated protein kinase (MAPK) pathways, which are controlled by protein phosphatases. What are the roles of Arabidopsis MAPK phosphatases and how they contribute to plant responses to pathogens? In Arabidopsis MAPKs MPK3, 4 and 6 have been identified in regulation of pathogen-induced pathways and MAPK phosphatases play important role in determination of MAPK activities and can affect signaling responses. We have identified Arabidopsis PP2C-type protein phosphatases AP2Cs as MAPK phosphatases, which interact with and inactivate MPK3, 4, 6. We found that PP2C type MAPK phosphatases dephosphorylate Arabidopsis MPK6 on phospho-T residue localized in the activation loop of the MAPK and inactivate its kinase activity. In plants MAPK activation leads to reprogramming of plant cellular activities, including changes in plant pathogen responses, such as ROS production and gene expression. Thus, we studied the role of plant MAPK phosphatases of PP2C type in regulation of MAP kinase activities, ROS production and pathogen-responsive gene expression during pathogen associated molecular pattern- or pathogen-induced stress. Our data show that AP2C controls plant immune responses and resistance to pathogens, emphasizing the important roles of PP2C type MAPK phosphatases in plant innate immunity.

Keywords: MAPK, PP2C, plant immunity, ROS, signaling

Poster #103. Glutamate fermentation co-product induces resistance to virus in Arabidopsis (Submission 464)
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Glutamate are produced by bacterial fermentation in the food industry. Through the purification of glutamate from fermentation, a large amount of co-product remains as industrial wastes. Therefore the co-product has been used for animal food and fertilizer, because it contains compounds from plant materials, nutrients for the fermentation, and bacterial components. Moreover, treatment with glutamate fermentation co-product (Copro) activates defense responses and enhances resistance to Pseudomonas syringae pv. maculicola in Arabidopsis (Igarashi et al., 2010 J. Phytopathol. 158:668-675). Here, it was found that Copro also confers enhanced resistance to Cucumber mosaic virus (CMV) and other virus species in Arabidopsis. Treatment of Copro a day before inoculation with CMV compared to buffer treatment as a control, the viral infection sites were specifically reduced in the inoculated leaves three days after inoculation. Copro in treatment a day after virus inoculation was also effective to suppress virus infection. From these results Copro induces Arabidopsis to confer enhanced resistance to CMV. Copro treatment induces the expression of plant defense-related genes including salicylic-acid inducible genes related to defense against pathogens. To test whether salicylic acid signaling pathway is required for Copro-conferred resistance, the salicylic acid signaling deficient eds5 mutant and nahG transgenic plants were treated with Copro followed by inoculation with CMV. Copro-treatment conferred enhanced resistance to CMV in eds5 and nahG plants comparable to wild-type plants. These results indicate that Copro-conferred virus resistance might be induced via novel pathways independent on salicylic acid and ethylene mediated signal transduction.

Keywords: induced resistance to virus, glutamate, signal transduction

Poster #104. Allelic variation in signaling by the disease resistance protein RPP1 (Submission 468)
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Through interactions with various phytopathogens, plants have evolved disease resistance (R) proteins that recognize pathogen virulence effector proteins and induce strong immune responses, including host cell death. These R proteins are generally modular in structure, with an N-terminal coiled-coil or Toll/Interleukin-1 Receptor (TIR) domain followed by a nucleotide-binding (NB) domain, and terminating in a leucine-rich repeat region. Despite the critical role of R proteins in immunity, the mechanisms by which these receptors detect pathogen
effectors and stimulate plant defenses remain poorly understood. In Arabidopsis, the R protein RPP1 (Resistance to Peronsopora parasitica 1) recognizes the effector protein ATR1 (Arabidopsis thaliana Recognized 1) from the oomycete pathogen Hyaloperonospora arabidopsidis. In this study, we examined two alleles of RPP1, from ecotypes Neiderzenz (NdA) and Wassilewskija (WsB). We noted that expression of the NdA allele of the TIR domain alone induced cell death in the absence of ATR1, while the WsB allele did not. Chimeric TIR proteins revealed sequences both within and outside of the TIR domain that contributed to this allele-specific activity. Additional biochemical characterization of the TIR domain will be discussed.

Keywords: resistance proteins, defense induction, protein-protein interactions

Poster #105. Rice Allelochemical Momilactone B Causes Abnormal Activation of Flavonoid Biosynthetic Pathway in Arabidopsis (Submission 481)
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Plants compete for resources including territories, nutrients, sunlight and water. Allelopathy is a battle for survival in which a secondary metabolite, allelochemical, is released to the environment to suppress the growth of other competitive plants. Momilactone B has been reported as a rice allelochemical secreted into the rhizosphere causing strong growth suppression of other plant species including Arabidopsis thaliana but weaker effect on themselves. However, the underlying mechanism of allelopathy is largely unknown.

In this study, we screened the SALK T-DNA insertion lines for altered sensitivity to momilactone B in Arabidopsis. We identified the transparent testa 6 (tt6) mutant exhibiting weaker growth restriction compared with the wild-type. Since TT6 belongs to the flavonoid biosynthetic pathway, we examined other related genes in the same pathway and found mutants tt3, tt7 and banyuls (ban) showing severe growth inhibition in response to momilactone B. These results suggest that momilactone B could manipulate the expression profiles of genes in the flavonoid pathway. Interestingly, BAN, which encodes anthocyanidin reductase and functions as a key negative regulator of flavonoid biosynthesis, is significantly down-regulated by momilactone B, indicating that it causes abnormal accumulation of flavonoids. Indeed, momilactone B induces the deposition of pigment in a whole seeding of the wild-type as observed in untreated ban mutant plants. Flavonoids have been shown to exert a variety of biological activities, and some chemicals, including catechin, quercetin and kaempferol, are secreted into the environment and function as allelochemicals. Therefore, we assume that momilactone B perturbs the flavonoid pathway to abnormally accumulate some flavonoids that halt the growth and/or development in Arabidopsis. Here, we will discuss the differential effect of momilactone B on rice and Arabidopsis.

Keywords: Allelopathy, Momilactone B, Flavonoids

Poster #106. From pathogen proteins to plant immune function (Submission 486)
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Plant pathogens are responsible for multi-billion dollar damages in natural and cultured ecosystems. One strategy most pathogenic organisms follow is the secretion of effector proteins that manipulate the host immune system to suppress defense responses. We work with the obligate biotrophic oomycete pathogen Hyaloperonospora arabidopsidis that causes downy mildew disease on Arabidopsis thaliana. Sequencing of the Hyaloperonospora arabidopsidis genome revealed the existence of approximately 140 candidate effector proteins likely to be involved in the alteration of plant defense processes to allow successful growth and reproduction.

There is considerable interest in finding host targets of pathogen effectors as this helps to shape our understanding of how those proteins work in planta to establish disease and how plants try to defend themselves against those attacks. Therefore, we have generated a yeast two-hybrid interaction network between Arabidopsis proteins and pathogen effector proteins from Hyaloperonospora arabidopsidis and the related potato pathogen Phytophthora infestans. This resulted in an experimentally determined plant-pathogen immune network in which most of the interactors have no previously described immune-system function.
Our results give insight into the organization and function of plant immunology and which part of the host cellular machinery might be required for effective defense against microbial infection. Moreover, findings will assist to shape a detailed mechanistic understanding of how pathogen effectors manipulate these complexes to increase pathogen fitness.

Keywords: plant immune system, plant pathogen interactions, Hyaloperonospora arabidopsidis

**Cell Biology**

**Poster #107. Subcellular and supracellular mechanical stress prescribes cytoskeleton behavior in Arabidopsis cotyledon pavement cells** (Submission 19)
Arun Sampathkumar¹, Pawel Krupinski², Raymond Wightman³, Pascale Milani⁴, Arezki Boudaoud⁴, Olivier Hamant⁴, Henrik Jonsson³, Elliot Meyerowitz¹

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Shape changes in animal cells and tissues are triggered by active mechanical deformations, and the resulting force pattern can in turn impact cellular events. Here, we investigate to what extent individual cell shape in Arabidopsis dictates the pattern of mechanical stresses and whether such stresses can control subcellular events in the context of pavement cells, which exhibit a typical jigsaw puzzle shape in leaf epidermis.

Using atomic force microscopy, we first established that cell shape is correlated with local mechanical reinforcements of the cell wall, matching the dominant internal microtubule organization and its predicted cellulose deposition pattern. Next, using a finite element-based mechanical model, we found that cell shape predicts a complex stress pattern in the outer cell wall. Using micromechanical perturbations, we showed that subcellular stresses control microtubule orientation and that this effect can be overridden by tissue level stresses. We also find that the microtubule response to stress is promoted by katanin-dependent microtubule severing, and conversely, that mechanical stress can increase severing activity.

These results demonstrate that pavement cell microtubules can respond to mechanical stress at a subcellular scale. We also show that subcellular events depend on a balance between cell and tissue shape-derived stresses. Last, we identify an amplification mechanism in which mechanical stress promotes the microtubule response to stress by increasing severing activity, within a positive feedback loop. This provides a scenario in which the robustness of microtubule behavior depends on shape-derived stresses across scales.

Keywords: Microtubule cytoskeleton, mechanical stress, cell wall, pavement cells

**Poster/Talk #108. The role of a mitochondrial membrane-bound ubiquitin protease in mitochondrial dynamics in Arabidopsis** (Submission 20)
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Research in our group is aimed at elucidating the regulatory mechanisms underlying the dynamic behavior of plant mitochondria and peroxisomes, essential organelles that are highly dynamic and act in concert in metabolic pathways crucial to energy production. We employed molecular genetics, cell biology, biochemistry and proteomics to identify proteins involved in the dynamics of these two types of organelles in Arabidopsis. Interestingly, a number of dual- or even triple-localized proteins have been found to be shared by peroxisomes and mitochondria, and sometimes even chloroplasts, for their division and proliferation. These shared factors include dynamin-related proteins DRP3 and DRP5B, DRP3's putative organelle anchor FISSION1 (FIS1), and the plant-specific protein Peroxisome and Mitochondrial Division factor 1 (PMD1) that seems to act independently from DRP3 and FIS1. Multiple levels of regulation are imposed on these proteins, resulting in the alterations of their function in both mitochondria and peroxisomes or in an organelle-specific manner. These regulatory mechanisms are just beginning to be elucidated in plants. We have shown that DRP3 protein phosphorylation can inhibit its function on both organelles, whereas the phospholipid cardiolipin stabilizes the protein complex of DRP3 only on mitochondria. Through a screen that combined bioinformatics and fluorescent fusion protein targeting analysis, we also identified an Arabidopsis mitochondrion-localized deubiquitinase, UBP27. Characterization of
UBP27 with respect to its membrane topology, enzymatic activity, and organelle morphology in both loss-of-function and overexpression lines will be presented. Our data suggest that UBP27 contributes to mitochondrial morphogenesis and promotes the recycling of DRP3 from mitochondria to the cytosol.

Keywords: mitochondria, peroxisomes, morphogenesis, dual function proteins, dynamin-related proteins, ubiquitin protease

Poster/Talk #109. A novel PVC-localized protein FREE1 is essential for vacuolar protein transport and vacuole biogenesis in Arabidopsis (Submission 26)
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Prevacuolar compartments (PVCs), identified as multivesicular bodies (MVBs) [1], are also late endosomes that mediate protein transport to vacuoles in both secretory and endocytic pathways of plant cells [2]. To uncover a full functional map of PVC, we have carried out PVC isolation and proteomics analysis for protein identification. One of these newly identified PVC proteins was termed as FREE1 (factors required for endosomal sorting 1). We aim to study the subcellular localization and function of FREE1 in Arabidopsis and have demonstrated that (1) loss of function of FREE1 by T-DNA insertion or by inducible RNAi caused seedling lethality to plant; (2) GFP-FREE1 fusion localized to PVC and fully complemented the lethal phenotype of free1 mutant; (3) free1 mutant lost central vacuole but instead accumulated lots of small vacuoles; (4) vacuolar protein transport was disrupted by FREE1 depletion. Taken together, these preliminary results showed that FREE1 was essential for vacuolar protein transport and central vacuole formation in Arabidopsis. To further understand the underlying mechanism of FREE1 function, we are performing yeast two hybrid screening of the Arabidopsis cDNA library and pull-down analysis using FREE1 as bait to identify FREE1 interaction partners for further characterization that are related with PVC-vacuole traffic [3] using a combination of transient expression, immunogold electron microscope (EM), biochemical, and genetic approaches. Here we will present an update on this study. Supported by grants from the Research Grants Council of Hong Kong (CUHK465112, 466613, CUHK2/CRF/11G and AoE/M-05/12).

References:

Keywords: Arabidopsis FREE1 protein, Prevacuolar compartment, Vacuolar protein transport, Vacuole biogenesis

Poster #110. Molecular characterization of Sar1 proteins in Arabidopsis (Submission 27)
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Secretory proteins traffic from the endoplasmic reticulum (ER) to Golgi apparatus via coat protein complex II (COPII) vesicles. Coat protein complex II, which consists of five cytosolic components (Sar1, Sec23-24, and Sec13-31), is responsible for COPII vesicle formation. Sar1, a Ras-related GTPase, is recruited to the ER membrane and initiates COPII vesicles formation by recruiting inner coat proteins Sec23-24. In mammal, Sar1B seems to be specialized to transport large cargos as mutations in Sar1B affect the secretion of large cargo chylomicrons [1]. However, little is known about COPII formation and Sar1 function in plants. The Arabidopsis genome contains five Sar1 homologues, in which AtSARA1a and AtSARA1b were shown to exhibit different
effects on α-amylase secretion [2]. However, the functional heterogeneity of AtSar1 homologues on cargos ER export and plant development remained unresolved. Towards this goal, we have carried out studies to illustrate the distinct functions of various AtSar1 homologues in plants. We have generated transgenic Arabidopsis cell lines and plants expressing GFP-tagged AtSar1 homologues, as well as specific AtSar1 antibodies for subcellular localization studies. We have tested and established an in vitro COPII budding system to be used to test the possible effects of AtSar1 homologues on cargos ER export and COPII formation. We have used homology modeling and FRET analysis to study and identify AtSar1 protein interaction partners in Arabidopsis. Finally, we have also tested and confirmed the distinct functions of AtSar1 homologues in plants. Here we will present an update about this study. Supported by grants from the Research Grants Council of Hong Kong (CUHK466011, 465112, 466613, CUHK2/CRF/11G and AoE/M-05/12).

Reference:

Keywords: COPII, AtSar1, In vitro COPII budding

Poster #111. Rab7 activation by the MON1-CCZ1 complex is essential for PVC-to-vacuole trafficking and plant growth in Arabidopsis

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Rab GTPases serve as multifaceted organizers in mediating vesicle trafficking. Rab7, a member of the Rab GTPase family has been shown to perform various essential functions in endosome and endosome to lysosome traffic in mammalian systems. The Arabidopsis genome encodes eight putative Rab7 homologs, however the detailed function and activation mechanism of Rab7 in plants remain unknown. Here we demonstrate that Arabidopsis RABG3f, a member of plant Rab7 small GTPases localizes to prevacuolar compartments (PVCs) and the tonoplast. The proper activation of Rab7 is essential for both PVC-to-vacuole trafficking and vacuole biogenesis. Expression of a dominant negative Rab7 mutant (RABG3fT22N) induces the formation of enlarged PVCs and affects vacuole morphology in plant cells. We also identify Arabidopsis MON1 and CCZ1 homolog proteins as a dimeric complex that functions as the Rab7 GEF. MON1-CCZ1 complex also serves as the Rab5 effector to mediate Rab5-to-Rab7 conversion on PVCs. Loss of functional MON1 causes the formation of enlarged Rab5-positive PVCs that are separated from Rab7-positive endosomes. Similar to the dominant negative Rab7 mutant, the mon1 mutants show pleiotropic growth defects, exhibit fragmented vacuoles and affect vacuolar trafficking [1]. Our on-going studies reveal that Rab7 activation may also play a role in male gametophyte development and likely the autophagic pathways in Arabidopsis [2, 3]. Here we will present an update about our study on Rab7 activation and function in Arabidopsis. Supported by grants from the Research Grants Council of Hong Kong (CUHK466011, 465112 and 466613, CUHK2/CRF/11G and AoE/M-05/12).

References:

Keywords: Rab7 activation, MON1-CCZ1 complex, PVC-to-vacuole trafficking, plant growth, male gametophyte development, autophagic pathways
Poster/Talk #112. Tubulin phosphorylation by NIMA-related kinases is involved in cell growth and division (Submission 41)
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Microtubules are highly dynamic structures controlling spatiotemporal pattern of cell growth and division. Microtubule dynamics are regulated by reversible protein phosphorylation involving both protein kinases and phosphatases. However, phosphorylation-dependent control of microtubule organization is not well understood. NIMA-related kinases (NEKs) are a family of eukaryotic protein kinases that are homologous to Never in mitosis A (NIMA) of Aspergillus nidulans. In fungi and animal cells, NEKs regulate microtubule-related mitotic events such as centrosome separation and spindle formation. Although plant NEK function remains obscure, our recent studies revealed that plant NEKs regulate both cell elongation and cell division via microtubule organization.

Among seven NEK members of Arabidopsis thaliana, NEK6 plays the central role in the regulation of cell growth and microtubule organization. NEK6 interacts with other NEK members, phosphorylates tubulin, and suppresses cortical microtubule stabilization. In addition, plant NEK members redundantly regulate the activity and orientation of cell division. The induction system of Arabidopsis NEKs showed that NEKs phosphorylates tubulin in vivo to regulate cell growth. In addition, tubulin phosphorylation sites were successfully identified by LC-MS/MS analysis and in vitro kinase assay. Together with previous results, tubulin phosphorylation by NEK6 may promote microtubule dynamics during cell growth and division.

Keywords: cell elongation, cell division, kinase, microtubule, tubulin

Poster #113. Abscisic acid induces ectopic outgrowth and cortical microtubule reorganization in epidermal cells of Arabidopsis thaliana (Submission 42)
Shogo Takatani1, Takashi Hirayama2, Takashi Hashimoto3, Taku Takahashi1, Hiroyasu Motose1
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Plant cells change their growth in response to external stimuli during adaptation to the environment. Phytohormones play pivotal roles in this process. However, little is known about mechanisms for cell morphogenesis in response to phytohormones. Here, we report that abscisic acid (ABA) induces ectopic outgrowth of epidermis. Seedlings of Arabidopsis thaliana were germinated and grown in the presence of ABA. ABA-treated seedlings developed ectopic protrusions in epidermal cells of hypocotyls and cotyledons. One protrusion was formed in the middle of each epidermal cell. In hypocotyls, two kinds of epidermal cell files are arranged alternately into the non-stoma cell file and the stoma cell file. Ectopic protrusions were restricted to the non-stoma cell files, suggesting the difference in the responsiveness to ABA between the two cell files. The ABA-induced ectopic outgrowth was suppressed in ABA insensitive mutants, whereas it was enhanced in ABA hypersensitive mutants. Interestingly, the ectopic outgrowth was also suppressed in microtubule-related mutants.

In addition, cortical microtubules were reorganized and depolymerized in the presence of ABA. In hypocotyls, two kinds of epidermal cell files are arranged alternately into the non-stoma cell file and the stoma cell file. Ectopic protrusions were restricted to the non-stoma cell files, suggesting the difference in the responsiveness to ABA between the two cell files. The ABA-induced ectopic outgrowth was suppressed in ABA insensitive mutants, whereas it was enhanced in ABA hypersensitive mutants. Interestingly, the ectopic outgrowth was also suppressed in microtubule-related mutants. In addition, cortical microtubules were reorganized and depolymerized in the presence of ABA. These results suggest that ABA signaling induces ectopic outgrowth in epidermal cells by microtubule reorganization.

Keywords: abscisic acid, microtubule, cell growth

Poster #114. Vacuolar Sorting Receptors Are Required for Vegetative Vacuole-Mediated Physiological Functions in Arabidopsis thaliana (Submission 44)
Tadashi Kunieda1, Noriyuki Hatsugeta1, Tomoo Shimada1, Maki Kondo2, Mikio Nishimura2, Ikuko Hara-Nishimura1
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Plant vacuole is a multifunctional organelle, which is involved in cell growth, tissue senescence, and the stress and defense responses via turgor pressure regulation, degradation, storage of metabolites and proteins, and so on. These vacuolar functions should be maintained with a number of vacuolar proteins. VACUOLAR SORTING RECEPTOR (VSR) is identified as a key factor for intracellular trafficking of the vacuolar proteins. VSR plays a role of sorting de-novo synthesized proteins at Golgi apparatus to deliver them to vacuoles. Extensive studies of VSR have been done with protein storage vacuoles (PSV). However, the physiological functions of VSR in
vegetative vacuoles remain to be cleared. There are seven VSR homologues (VSR1-7) in Arabidopsis thaliana. VSR1, VSR3 and VSR4 are highly expressed in vegetative tissues, suggesting that they function in protein sorting to vegetative vacuole. We isolated a mutant lacking both VSR3 and VSR4 genes from fast neutron mutagenized seeds. This double knockout mutant showed a delay of biosynthesis of sinapoyl malate, which is a vacuolar substance involved in UV resistance, although each single mutant did not. This phenotype was caused by missorting of a vacuolar protein, SINAPOYLGLUCOSE1 (SNG1), to extracellular space. This result indicates that VSR3 and VSR4 are redundantly required for trafficking of SNG1 to vegetative vacuole. The vsr3 vsr4 mutant facilitated leaf senescence under the growth condition lacking carbon source and impaired immune response against avirulent bacteria, suggesting that senescence and immune response are closely related to vacuolar functions. Interestingly, the vsr3 vsr4 mutant rarely caused organ fusion among vegetative tissues including leaves, stems and flower buds. This implies that a vacuolar enzyme recognized with VSRs may function as cutinase in extracellular space. These abnormal phenotypes were complemented by expressing VSR4 under control of the native promoter. These results suggest that VSR3 and VSR4 are required for a variety of functions of vegetative vacuole via proper protein sorting. Additionally, VSR1 has been reported to preferentially functions in transport of seed storage proteins to PSV. As VSR1, our result showed that VSR3 and VSR4 also function in vacuolar transport of seed storage proteins to PSV. Together, these results indicate that VSR3 and VSR4 play a role not only in vegetative vacuole but also in PSV.

Keywords: Vacuole, Vacuolar Sorting Receptor (VSR), Sinapoyl malate, SINAPOYLGLUCOSE1 (SNG1), Leaf senescence, immune response, organ fusion

Poster/Talk #115. Proteomic analysis reveals a framework in endomembrane compartments associated with immunity (Submission 48)
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The plasma membrane forms the primary interface through which plant cells sense and interact with the environment. Thus, exocytosis and endocytosis, processes by which plasma membrane composition is regulated, are critical components of the plant’s response to a changing environment, including during biotic interactions. To identify proteins that regulate and traffic through the exocytic and endocytic pathway before and during biotic interactions, we performed in depth proteomic screening, with a versatile simple method, of seven different compartments including: Clathrin coated vesicles, Golgi, post-Golgi, vacuole and endosomal compartments in Arabidopsis thaliana. We identified a total of 852 proteins specifically enriched after immunopurification representing the proteomes of seven endomembrane compartments. Comparative analysis revealed numerous described and potential regulators of the endomembrane system, some of which were shared among all endomembrane proteomes. One example is the ARF-GEF MIN7, a known target of the Pseudomonas syringae effector HOPM1. Our proteomic data can be used to predict the localization of proteins identified, as demonstrated with members of the important PRA1 endomembrane regulator family. Our data also shed new light on the composition of the vital tethering complexes, TRAPPII and GARP, as well as elucidating fundamental differences between the regulation of RAB5 GTPases in A.thaliana. Furthermore, we found components of the MAPK cascade present in several endosome proteomes suggesting a role of these compartments in defense signaling. We will discuss our recent results addressing the possible role of endosomes in bacterial flagellin triggered immunity and the proteomic changes in ARA7 endosomes after flagellin treatment. Thus, our data provides a proteomic framework of exocytic and endocytic trafficking with roles in plant immunity.

Keywords: Endosome, proteomics, ARA7, ARA6, Clathrin, flg22, FLS2

Poster #116. Fluorescent sensors for activity and regulation of the nitrate transceptor CHL1/NRT1.1 and oligopeptide transporters (Submission 53)
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To monitor nitrate and peptide transport activity in vivo, we converted the dual-affinity nitrate transceptor CHL1/NRT1.1/NPF6.3 and four related oligopeptide transporters PTR1, 2, 4 and 5 into fluorescent activity sensors (NiTrac1, PepTrac). Substrate addition to yeast expressing transporter fusions with yellow fluorescent protein and mCerulean triggered substrate-dependent donor quenching or resonance energy transfer. Fluorescence changes were nitrate/peptide-specific, respectively. Like CHL1, NiTrac1 had biphasic kinetics. Mutation of T101A eliminated high-affinity transport and blocked the fluorescence response to low nitrate. NiTrac was used for characterizing side chains considered important for substrate interaction, proton coupling, and regulation. We observed a striking correlation between transport activity and sensor output. Coexpression of NiTrac with known calcineurin-like proteins (CBL1, 9; CIPK23) and candidates identified in an interactome screen (CBL1, KT2, WNKinase 8) blocked NiTrac1 responses, demonstrating the suitability for in vivo analysis of activity and regulation. The new technology is applicable in plant and medical research.

Keywords: nitrogen, transporter, fluorescent proteins, structural

Poster #117. PES plays an essential role in ribosomal RNA processing and ribosome biogenesis in plants. (Submission 55)
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Pescadillo (PES) is a multifunctional protein involved in embryonic development, ribosomal biogenesis, cell proliferation, and gene transcription in yeast and metazoans. In this study, we characterized cellular functions of plant PES in Nicotiana benthamiana, Arabidopsis, and tobacco BY-2 cells. A GFP fusion protein of PES is predominantly localized in the nucleolus, where its localization requires the N-terminal domain of PES. Silencing of plant PES led to growth arrest and acute cell death. PES interacts with plant homologs of BOP1 and WDR12 in the nucleolus, which are also nucleolar proteins involved in ribosome biogenesis of yeast and mammals. PES, BOP1, and WDR12 cofractionated with ribosome subunits. Depletion of any of these proteins led to defective biogenesis of the 60S ribosome large subunits and disruption of nucleolar morphology. PES-deficient plant cells also exhibited delayed maturation of 25S ribosomal RNA and suppressed global translation. During mitosis in tobacco BY-2 cells, PES is associated with the mitotic microtubules, including spindles and phragmoplasts, and PES deficiency disrupted spindle organization and chromosome arrangement. Collectively, these results suggest that plant PES has an essential role in cell growth and survival through its regulation of ribosome biogenesis and mitotic progression.

Keywords: Pescadillo, Ribosome biogenesis, BOP1, WDR12, PeBoW complex, mitotic progression

Poster #118. Unique function of poly(A)-specific ribonuclease of Arabidopsis (Submission 76)
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Post-transcriptional regulation is one of the most important gene regulatory steps in eukaryotes. Recent studies have shown that eukaryotes have developed elaborated mechanisms to control mRNA stability. In brief, poly(A) tract at 3' end of RNA is the major determinant of the RNA stability. Basically, poly(A) tract stabilizes cytoplasmic mRNA. Eukaryotes have three different types of poly(A) removal factors, deadenylase, namely CCR4-NOT-CAF complex, PAN2-PAN3 complex, and poly(A) specific ribonuclease (PARN). By contrast, poly(A) tract destabilizes rRNA, tRNA, and other functional RNAs in eukaryotes. Plant organellar mRNA is also destabilized by poly(A) addition although their regulations have not been fully understood yet. Disruptant mutation of Arabisopsis PARN causes lethality while a weak allele, named ahg2-1, with a reduced expression level of PARN, exhibits abnormal responses to stress-related plant hormones (ABA and SA), and a
dwarf phenotype, suggesting that PARN has important functions in plants. To address the role of PARN in Arabidopsis, we took genetic and molecular biological approaches. All the suppressor mutants for ahg2-1 have defects in a gene (named AGS1) encoding a poly(A) polymerase (PAP), strongly suggests that the poly(A) status of certain RNA(s) is the determinant of the ahg2-1 mutant phenotype. Microarray analyses revealed that most of the mitochondrial mRNAs are abnormally polyadenylated and more accumulated in ahg2-1. We also demonstrated, 1) some of mitochondrial protein complexes are unstable in ahg2-1, 2) both AHG2/PARN- and AGS1-GFP fusion proteins localize at mitochondria, 3) removal of putative N-terminal transit sequences of these proteins reduced their in vivo functions. These data suggest that, surprisingly in Arabidopsis, PARN directly regulates the poly(A) status of mitochondrial mRNA cooperating with, a PAP, AGS1. Given two independent genome possessing organelles, mitochondrion and plastid, plants should have developed a unique system to regulate the gene expressions in these organelles, and presumably PARN had been specialized for mitochondrial mRNA regulation. Now we are trying to deduce the regulatory mechanisms for AHG2 and AGS1 by analyzing their interacting proteins.

ref: Hirayama et al., Nature Commun, 4, #2247, 2013

Keywords: poly(A)-specific ribonuclease, poly(A) polymerase, mitochondria, post-transcriptional regulation, hormonal response

Poster/Talk #119. IMPAIRED TRAFFIC TO TONOPLAST5 (ITT5) encodes a novel protein of unknown function involved in vacuole bulb formation. (Submission 91)
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The plant vacuole is an essential organelle that is critical for maintenance of turgor and the storage of a multitude of molecules including nutritional proteins, defense molecules against pathogens, and other secondary metabolites. Two types of plant vacuoles exist, the protein storage vacuole (PSV) and the lytic vacuole. The PSV has a neutral pH and is established during embryonic development as the storage compartment for seed storage proteins. The lytic vacuole has an acidic pH and is the predominant compartment in vegetative cells. The mechanisms for biogenesis of protein storage vacuoles in embryos and lytic vacuoles in vegetative tissues are poorly characterized.

Vacuoles are dynamic compartments with constant fluctuations and formation of transient structures such as trans-vacuolar strands (TVS) and bulbs. Bulbs are invaginations of the vacuolar membrane that have been proposed as sites for membrane or protein degradation or as reservoirs for membranes that may be needed for cell growth. Both PSVs and lytic vacuoles have been shown to form bulb structures. Even though bulbs are dynamic structures and associated with the tonoplast, it appears as if there is some specificity in the accumulation of certain proteins to the bulbs.

We recently carried out a screen for mutants with abnormal trafficking to the vacuole or aberrant vacuole morphology (Zheng et al, 2014 Mol. Plant). We have characterized impaired trafficking to tonoplast 5 (itt5), a mutant in Arabidopsis that contains increased numbers of bulbs when compared to the control. Analysis of the itt5 phenotype indicates that bulb formation is developmentally regulated and responds to environmental signals. A map-based cloning approach combined with next generation sequencing was used to identify the ITT5 locus. We present evidence that ITT5 corresponds to a novel protein of unknown function that is specific to plants and localizes to the cytosol. Characterization of itt5 is being used to determine the function and cellular dynamics of vacuolar bulbs in Arabidopsis.


Keywords: vacuoles, membrane fusion, bulbs, endomembrane system

Poster #120. The role of HOPS tethering complex in plant vacuole fusion (Submission 92)
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The vacuole is an essential and dynamic organelle involved in plant development, growth and responses to
environmental stress. However, the molecular mechanisms that contribute fusion of vacuolar membranes are not fully understood. A vacuolar SNARE [soluble N-ethylmaleimide-sensitive factor (NSF) attachment receptor] complex composed by SYNTAXIN OF PLANTS22 (SYT22), SYP51/52, VESICLE-ASSOCIATED MEMBRANE PROTEIN727 (VAMP727), and VT11 is important to mediate membrane transport from pre-vacuolar compartment (PVC) to the vacuole. We recently identified a role for VT11 in vacuole fusion both during plant development and during the dynamic changes of vacuole morphology in guard cells (Zheng et al, 2014 Mol. Plant). Thus, two vt11 mutant alleles, zig1 and impaired traffic to tonoplast3 (itt3), showed multiple vacuoles in root and hypocotyl cells, reduced gravitropic responses and abnormal stomata movements. Moreover, treatment with inhibitors of Phosphatidylinositol 3-kinase (PI3K) induced vacuole fusion in roots and guard cells. Therefore, both SNAREs and phosphoinositides are key regulators of vacuole membrane fusion. Membrane fusion is necessary for biogenesis and maintenance of endomembrane compartments in all eukaryotes. In yeast, fusion of endocytic compartments requires SNAREs, Rab GTPases, phosphoinositides and the tethering complexes HOPS and CORVET. HOPS [HOmotypic fusion and vacuole Protein Sorting] regulates vacuole fusion and biogenesis by capturing, chaperoning and proofreading the fidelity of the SNARE complex. The HOPS complex in plants has not been characterized yet, but at least one member, VACUOLESS1 (VCL1), has been shown to be essential for the biogenesis of the vacuole. Our goal is to determine the role of HOPS in plant vacuole fusion, including its interaction with SNAREs and phosphoinositides. Initial characterization of the Arabidopsis HOPS complex, with particular attention to the putative AtVPS33 homologue will be presented.


**Keywords:** vacuole, fusion, membrane, tethering, complexes, HOPS

**Poster #121. Cellular remodelling and protein storage vacuole biogenesis during vegetative to embryonic developmental transitions in Arabidopsis thaliana** (Submission 107)
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Flowering plants have two types of vacuoles; lytic vacuoles (LV) are present in most vegetative cells while protein storage vacuoles (PSV) are commonly observed in embryonic cells. During the maturation phase of embryo development, LV are replaced by PSV which specialize in sequestering minerals and seed storage proteins. During seed germination, PSV contents are mobilized and PSV are once again replaced by LV. In Arabidopsis thaliana, the study of PSV presents technical challenges due to small seed size and difficulties in isolating embryos from maternal tissues at earlier stages of embryogenesis. The purpose of this study is to visualize the dynamics of cellular remodelling and the biogenesis of PSV in Arabidopsis. To achieve this, we generated several Arabidopsis lines expressing fluorescent markers for different organelles of the secretory pathway or autophagy route. Using confocal microscopy, the fluorescently-labeled organelles are being examined in developing embryos as well as in leaves reprogrammed by over-expression of the LEAFY COTYLEDON2 (LEC2) transcription factor. LEC2 is sufficient to activate seed gene expression and thus causes vegetative organs to exhibit embryonic traits, including the appearance of PSV-like organelles. In dexamethasone-induced 35S:LEC2-GR plants, a massive cellular reorganization is observed in leaf epidermal cells. However, the cellular response to LEC2 is asynchronous within a tissue. Thus, it appears that the transition between vegetative and embryogenic characteristics occurs at different stages in individual cells. In induced leaf cells, the LV undergoes dramatic remodelling at high speed with an increased number of transvacuolar strands. These cells also accumulate numerous swollen chloroplasts containing large starch granules. In addition, oil bodies accumulate in the increasing volume of cytoplasm. Strikingly, a subunit of the vacuolar ATPase (VHA-a3-mRFP) and tandem-pore potassium channel (TPK1-eGFP) proteins, which reside on the LV tonoplast, are internalized in the PSV lumen. In developing embryos, LV appear to be replaced by PSV during late torpedo to bent cotyledon stages. Work is ongoing to characterize marker lines in inducible LEC2 lines and corroborate these results in native tissues. Subsequently, these will be applied to study the biogenesis of PSV in both systems.

**Keywords:** Arabidopsis thaliana, cellular reprogramming, confocal microscopy, developmental transition, LEAFY
**Poster #122. p24 cargo receptors CYB1 and CYB2 are required for intracellular trafficking of a myrosinase-associated protein GLL23 (Submission 118)**

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For a cell to function properly and survive, secretory proteins have to be delivered to the intracellular location at which they function. Protein transport specificity in the secretory pathway is achieved in part through the use of cargo receptors, which recruit specific protein cargoes into secretory vesicles. Emerging evidence suggests that proteins belonging to a p24 family play an important role in secretory cargo selection. These small ~20-25 kDa transmembrane proteins are localized to the early secretory pathway. Their short C-terminal cytosolic tails bind to the COPI and COPII subunits, whereas their luminal side is postulated to act as a cargo receptor. The subset of possible secretory cargoes of p24s has been identified in yeast and animal cells; however, until recently, no potential cargoes have been described for plants. Also, with no prior identification of plant p24 mutants, it was not clear if disruption of plant p24 proteins would affect endomembrane trafficking and cell function.

Our recent study (Jancowski et al., 2014, Plant J. 77:497-510) has demonstrated for the first time that the mutation of Arabidopsis ER and Golgi localized p24 protein CYTOPLASMIC BODIES1 (CYB1, At1g57620) affects the ER-Golgi export of a myrosinase-associated GDSL-like lipase GLL23, which is implicated in plant defense. In the absence of functional CYB1, GLL23 accumulates within cyb bodies - small (~ 2-3 um) spindle-shaped compartments associated with the peripheral ER. These results suggest that CYB1 is required for ER export of GLL23, most likely acting as its cargo receptor. In the present study, we have shown that another member of the p24 family, CYB2, is also required for intracellular trafficking of GLL23. In the cyb2 mutant, GLL23 accumulates within ER-associated cyb compartments. As p24 proteins are known to form hetero-oligomeric complexes required for their proper intracellular location and function, CYB1 and CYB2 most likely act together to ensure efficient ER to Golgi export of GLL23. The subcellular location of CYB2 and its potential interactions with CYB1 and GLL23 are currently under investigation. Our results support the role of p24 proteins in the spatial isolation of the myrosinase-glucosinolate and ER body defense system components.

Keywords: p24 proteins, ER export, GDSL-like lipase, myrosinase-associated protein

**Poster #123. Profilin modulates the stochastic dynamic behavior of actin filaments in live epidermal cells (Submission 158)**

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The actin cytoskeleton functions in a variety of cellular processes, such as cell division, cytoplasmic streaming, vesicle trafficking, as well as response to biotic stresses. The stochastic dynamic behavior of actin filaments plays a pivotal role in coordinating these physical cellular processes. Using state-of-the-art imaging modalities and a robust collection of quantitative tools, we developed a model for the turnover and incessant remodeling of actin filaments in the cortical cytoskeletal array of plant epidermal cells. Through initial tests of the model, we demonstrated that Actin Depolymerizing Factor4 (ADF4) facilitates turnover of actin filaments by its severing activity and Capping Protein (CP) regulates the availability of actin filament barbed ends in live cells. However, most of the actin in plant cells exists as a monomer (G-actin) rather than in filaments. Profilin (PRF) forms a 1:1 complex with G-actin and is the most abundant G-actin binding protein in plants. Based on previous biochemical analyses, PRF-bound G-actin adds to the barbed end of actin filaments to promote elongation but suppresses spontaneous nucleation and subunit addition at pointed ends. How exactly PRF modulates the properties of G-actin and contributes to stochastic actin dynamics in living cells warrants further study. Here, we dissect the contribution of PRF1 to actin organization and dynamics during axial cell expansion by analyzing the behavior of individual filaments in the cortical array of living Arabidopsis thaliana epidermal cells. We found that reduced PRF1 levels result in increased overall actin dynamity, but significantly decreased the actin filament elongation rate and maximum filament length. In addition, we observed that the frequency of nucleation events in prf1 mutants was dramatically decreased. Our data provide compelling evidence that PRF1 coordinates the stochastic
dynamic properties of actin filaments during plant cell expansion. However, the present data for PRF1 modulating actin stochastic dynamics in vivo do not match our expectations based on the biochemical properties of PRF1. Therefore, we assume that there must be novel mechanisms for PRF1 regulation or undiscovered synergies with other actin-binding proteins that require further evaluation.

Keywords: cytoskeleton, live cell imaging, actin dynamics, actin-binding proteins, signaling

Poster/Talk #124. Domain-specific lignification of Arabidopsis protoxylem is mediated by laccase-catalyzed deposition. (Submission 190)
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Plants precisely control lignin deposition in specific secondary cell wall domains during protoxylem tracheary element (TE) development. Live cell imaging and the inducible-VASCULAR NAC DOMAIN7 (VND7) protoxylem TE differentiation system were used to test if the subcellular localization of monolignol biosynthetic, transporter, or apoplastic polymerization components could account for the precise lignin deposition in spiral wall thickenings. Enzymes of the monolignol biosynthetic pathway, CINNAMATE 4-HYDROXYLASE and CINNAMOYL CoA REDUCTASE1 tagged with fluorescent proteins, were localized to the endoplasmic reticulum and throughout the cytoplasm, respectively. Several ATP-binding cassette (ABC) transporters localized evenly to all plasma membrane domains during protoxylem TE differentiation. Unrestricted mobility of monolignols throughout plant organs was observed when fluorescently tagged monolignols were exogenously supplied. Monolignol incorporation into lignin occurred specifically in secondary cell wall domains and was dependent on LACCASE4 and LACCASE17 function. In contrast to the broad distribution of biosynthetic and putative transporter components, LACCASE4 was precisely localized to secondary cell wall thickenings. Our data support a model wherein monolignols are highly mobile once exported to the cell wall, and in which monolignol polymerization in specific cell wall domains is determined by the specific localization of cell wall laccases.

Keywords: Protoxylem, Lignin, Laccase, Arabidopsis thaliana

Poster #125. THE IMPORTANCE OF TONOPLAST AQUAPORINS IN ARABIDOPSIS THALIANA GERMINATION AND SEEDLING GROWTH (Submission 198)
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Tonooplast Intrinsic Proteins (TIP) are aquaporins transporting water and small uncharged molecules across the tonoplast. Expression of the 10 TIP isoforms of Arabidopsis thaliana is both developmentally and spatially regulated. Little is known about how TIP are sorted to the tonoplast or their roles in germination and seedling growth, specifically in water stress conditions. In arabidopsis embryos, TIP3 isoforms are uniquely found at both the plasma membrane and tonoplast. It is suspected that their C-terminal motif is responsible for plasma membrane localisation. By using pharmacological approaches, we have established that TIP3 trafficking is insensitive to brefeldin A treatment and that sorting to the tonoplast is independent of their C-terminal motif. We are studying whether targeting to both membranes is simultaneous or sequential using an inducible expression system. TIP3;1 and TIP3;2 are the only aquaporins present in maturing and germinating seeds. We therefore hypothesise that TIP3 may be key to efficient water uptake in these developmental stages. To test this hypothesis we have developed assays to measure germination and seedling growth in varying levels of water stress and of TIP3 up- or down-regulation. Homozygous transgenic lines constitutively overexpressing TIP3;1 display significantly decreased efficiency in germination and seedling growth compared to wild type plants in increased water stress conditions. However, expression of TIP3;2 and the vegetative plasma membrane aquaporin PIP1;2 under the TIP3;2 promoter, increased efficiency of germination and seedling growth in water stress conditions. This indicates that
manipulation of aquaporin expression may increase tolerance of A. thaliana to water stress during these developmental stages.

Keywords: Aquaporins, TIP3, plasmamembrane, tonoplast, targeting, germination, growth, water, stress

Poster #126. Reduction and increase in TAM activity differentially affect chromosomal morphology and the subnuclear localization of ASY1 in prophase I but both lead to dyad formation (Submission 205)
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Cyclins are known regulators of both meiotic and mitotic cell cycle progression in diverse organisms. In Arabidopsis, loss-of-function mutations in the A-type cyclin CYCA1;2/TARDY ASYNCHRONOUS MEIOSIS (TAM) gene lead to the production of the abnormal meiotic product, dyad, via delayed progression through the first nuclear division and the absence of the second nuclear division. To investigate the effect of overexpression of TAM on meiosis, we generated Arabidopsis plants that harbored the ASK1:TAM transgene that ubiquitously increased TAM expression. T1 plants from five of six independent transformations produced dyads in male meiosis, indicating that the transgene had a dominant-negative effect. To understand how overexpression of TAM produced the same meiotic product as the loss-of-function mutants, we conducted detailed analysis of spread prophase-I chromosomes in the wild type (WT), tam-1, and an ASK1:TAM line. We found that the synapsed chromosomal regions in zygotene were thinner in ASK1:TAM than in the WT (t-test, p < 0.01) while such regions in tam-1 were not statistically different from those in the WT. However, the synapsed regions from zygotene through early diplotene in ASK1:TAM were consistently thicker than those in the WT. The synapsed regions in tam-1, on the other hand, appeared only thicker than the WT in zygotene. We also found that the centromeres in tam-1 meiocytes often formed a tight cluster at the pachytene and early diplotene stages. Single centromere clusters in tam-1 occurred in approximately 37% of pachytene meiocytes and 51% of early-diplotene meiocytes at 22[°]C, and 39% of pachytene meiocytes and 79% of early-diplotene meiocytes at 28[°]C. The meiocytes of WT and the ASK1:TAM rarely had such a single centromere cluster. Because the levels of TAM affects chromosomal morphology in prophase I, we examined the localization of the chromosomal axis component ASY1 in male meiocytes in the WT, tam-1, tam-2, and ASK1:TAM by immunolocalization. During pachytene, ASY1 was found to be colocalized with the chromosomes in the WT, tam-1, and tam-2, but ASY1 were also found abundantly in the nucleoplasm in addition to the colocalization with the chromosomes in ASK1:TAM. These observations suggest that the reduction and increase in TAM activity differentially affect chromosomal morphology and the subnuclear localization and/or abundance of ASY in prophase I but both negatively affect cell cycle progression, leading to dyad formation.

Keywords: meiosis, cell cycle progression, chromosome morphology, subnuclear localization, cyclin, loss of function, overexpression, synapsis

Poster #127. CER11/CTD Phosphatase Like 2 (CPL2) is Required for Secretory Trafficking of Cuticular Wax and Seed Coat Mucilage in Arabidopsis thaliana (Submission 231)
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Secretory trafficking is highly conserved in all eukaryotic cells. It is required for secretion of proteins as well as extracellular matrix components. In plants, export of cell wall polysaccharides, such as pectin and hemicellulose, involves the secretory pathway. It has also been hypothesized that cuticular wax is transported from the endoplasmic reticulum to the plasma membrane by vesicular trafficking via the Golgi apparatus. However, the detailed mechanism of the secretion of cell wall components and cuticular wax is still not well understood. In this study, we characterize the ciceriferum 11 (cer11) mutant, which exhibits dwarfism and a bushy stature, reduced stem cuticular wax deposition, aberrant seed coat mucilage extrusion, delayed secondary cell wall columella formation, as well as a block in secretory green fluorescent protein (secGFP) secretion. The pleiotropic cer11 phenotype suggests that CER11 plays a role in secretory trafficking involved in the deposition of apoplastic matrix components, including cuticular wax, seed coat mucilage and cell wall constituents. Cloning of the CER11 gene revealed that it encodes a C-TERMINAL DOMAIN PHOSPHATASE LIKE 2 (CPL2) that interacts with a vacuolar
type H+-ATPase (V-ATPase) subunit C (VHA-C) in yeast and plants. V-ATPase has been shown to be involved in secretory trafficking. Therefore, we hypothesize that CER11/CPL2 influences secretion by determining V-ATPase activity through dephosphorylation of VHA-C.

Keywords: Secretory trafficking, Cuticular wax, Seed coat mucilage, Cell wall, CER11, CPL2

Poster #128. The microtubule plus end tracking Armadillo Repeat Kinesin 1 promotes microtubule catastrophe in Arabidopsis thaliana. (Submission 232)
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Microtubule dynamics are critically important for plant cell development. Here we show that the Arabidopsis thaliana ARMADILLO-REPEAT KINESIN 1 (ARK1) plays a key role root-hair tip growth by promoting microtubule catastrophe events. This destabilizing activity appears to maintain adequate free tubulin concentrations in order to permit rapid microtubule growth, which in turn is correlated with uniform tip growth. Microtubules in ark1-1 root hairs exhibited reduced catastrophe frequency and slower growth velocities, both of which were restored by low concentrations of the microtubule-destabilizing drug oryzalin. An ARK1-GFP fusion protein expressed under its endogenous promoter localized to growing microtubule plus ends and rescued the ark1-1 root-hair phenotype. Transient overexpression of ARK1-RFP increased microtubule catastrophe frequency. ARK1-fusion protein constructs lacking the N-terminal motor domain still labeled microtubules, suggesting the existence of a second microtubule-binding domain at the C-terminus of ARK1. ARK1-GFP was broadly expressed in seedlings but mutant phenotypes were restricted to root hairs, indicating that ARK1’s function is redundant in cells other than those forming root hairs.

Keywords: Root Hairs, Tip Growth, Microtubules, Cytoskeleton, Kinesin

Poster #129. Plastid Genome Instability Leads to Reactive Oxygen Species Production and Plastid-to-Nucleus Retrograde Signaling in Arabidopsis (Submission 244)
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Genomic stability is absolutely essential to ensure cell functions and transmission of the genetic material. In plants, the plastid genome is no exception as it encodes essential components of the photosynthetic apparatus. Nevertheless little is known about the direct consequences of plastid genome instability. Recently, it was reported that the plastid Whirly proteins, WHY1 and WHY3, and a specialized type-I polymerase, POLIB, protect against plastid genome instability in Arabidopsis thaliana. In the present study, we used ciprofloxacin, an organelle specific mutagen, and why1why3polIb-1 mutant line to evaluate the impact of DNA rearrangements in the plastid genome. First, we show that in plants treated with ciprofloxacin and in why1why3polIb-1 plants, plastid genome instability leads to increased production of reactive oxygen species (ROS). Then, using different light regimes, we demonstrate that the elevated ROS production cause the appearance of a yellow-variegated phenotype affecting the entire why1why3polIb-1 population. Furthermore, the elevated ROS production in why1why3polIb-1 leads to chloroplast-to-nucleus retrograde signaling and to modifications of nuclear expression patterns promoting acclimation to high light. Taken together, our results bring evidence of the importance of plastid genome stability to avoid imbalance in plastid redox state.

Keywords: Chloroplast, Genome Stability, Reactive Oxygen Species, Plastid-to-Nucleus Signaling

Poster #130. Integration of ROP signaling with cytoskeletal and cell wall systems during Arabidopsis trichome morphogenesis (Submission 269)
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Arabidopsis leaf trichomes are specialized branched cells that rely on strict spatial and temporal control of the microtubule and actin cytoskeleton systems to coordinate polarized cell elongation. Forward genetic screens have identified many players in cell shape control, including a complete ROP-based signaling pathway that leads to
actin filament polymerization. The guanine nucleotide exchange factor SPIKE1 generates ROP-GTP signals that positively regulate the heteromeric WAVE/SCAR regulatory complex, which in turn converts the ARP2/3 complex into a potent actin filament-nucleating machine. Despite thorough knowledge about the components and regulatory interactions of this pathway, an understanding of their cellular deployment and the influence of actin on cell wall properties and shape change has been slow to emerge. This knowledge gap is not unique to trichomes: in general, the function of cortical actin networks during cell morphogenesis is not known. Here we describe a new approach to cell shape control that integrates time-dependent analyses of actin networks, microtubules, and cell wall systems using combinations of light and electron microscopy. These data were used to parameterize a realistic finite element (FE) computational model that links cytoskeletal behaviors, regional control of the mechanical properties of the cell wall, and growth. The FE model made specific predictions concerning important cell wall heterogeneities that were experimentally validated. Live cell analyses of ARP2/3 revealed its full activation at the branch apex; however the actin-dependence of the growth process has nothing in common with tip growth. Instead the experimental and computational data both point to a system in which microtubules and cortical actin meshworks cooperate to define local and global cell wall properties that enable cell patterning to be completed at early stages of trichome morphogenesis.

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Keywords: cytoskeleton, computational modeling, actin, cell wall, microtubule, signaling

Poster #131. Plant exocyst complex in autophagy and defence (Submission 275)

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We have characterized exocyst complex in Arabidopsis and showed that eight predicted subunits form a complex which does participate in exocytosis and membrane recycling (for review see - Zarsky et al. 2013). Analyses of phenotypic deviations of Arabidopsis exocyst mutants clearly indicate exocyst complex functions in cell wall biogenesis, polarized cell expansion, cell division, PIN proteins recycling and polar auxin transport. As an important aspect of plant - microbes interactions biogenesis of membraneous contact zones between membranes/apoplasts of interacting partners is also dependent on exocytosis; exocyst is involved in the perifungal membrane biogenesis in mycorrhiza. It seems, that the multiplicity of EXO70 subunits in land plants (23 in Arabidopsis) is also a consequence of the competition with pathogens - e.g. EXO70B2 and EXO70H1 exocyst subunits are induced by MAMPs. Mutations in these subunits compromise defence interaction with microbes including a regular build-up of defensive papillae (Pecenkova et al. 2011). Recently we identified unexpected exocyst complex subunit EXO70B1 and interacting subunits participation in the regulation of autophagy-related GA-independent membrane trafficking to the vacuole directly from the ER (Kulich et al. 2013). In exo70b1 autophagy-compromised mutant spontaneous hypersensitive reaction is induced due to high salicylic acid accumulation. We will report on our new insights into exocyst functions and interactions. The exocyst complex involvement in the exocytosis and vacuolar traffic indicates a possibility that it may contribute to the coordination of endomembrane dynamics in plants.

This work is supported by the Czech Science Foundation grant GACR P305/11/1629


Keywords: secretory pathway, cell polarity, vesicle targeting, exocyst tethering complex, autophagy, defence, EXO70, salicylic acid
Poster #132. Mapping of Xylan Biosynthesis in the Golgi of Developing Xylem (Submission 294)
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¹University of British Columbia, Department of Botany, Canada, ²University of British Columbia, Department of Wood Science, Canada
Plant cells with thickened secondary cell walls (SCWs) provide structural support and play an important physiological role in growth and development. Our understanding of SCW development has been greatly improved by studies employing the Arabidopsis model system. However, this system is limited by the relatively small size and number of the developing vascular cells, and their depth within plant tissues. This limitation can be overcome by induction of the master transcription factor VND7, causing ectopic transdifferentiation into SCW containing protoxylem cells. A major component of the SCWs of dicots is the hemicellulose polysaccharide glucuronoxylan (GX). The glycosyltransferases IRX9 and IRX10 are thought to synthesize the GX backbone in the Golgi. To study GX synthesis, proIRX9:IRX9:GFP and proIRX10:IRX10:GFP constructs were generated and shown to restore wild-type height and vasculature to irx9 and irx10irx10L mutants, respectively. Following VND7 induction, reticulate (indicating ER localization) and donut-shaped punctae (characteristic of Golgi localization) fluorescent signals were detected. This suggests that both IRX9 and IRX10 are co-translationally inserted into the ER, as implied by their predicted topology. However, both glycosyltransferases are primarily localized to the Golgi, as expected for hemicellulose biosynthetic enzymes. Ultimately, IRX9 and IRX10 must be retained in the Golgi while the GX cargo is deposited to developing SCW thickenings. The GX antibody LM10, in conjunction with transmission electron microscopy, should provide a useful tool for elucidating the role of the Golgi as cell wall carbohydrates like GX are synthesized.

Keywords: Golgi, Cell Wall, Xylan

Poster #133. Arabidopsis Dynamin-Related Proteins, DRP2A and DRP2B, function coordinately in post-Golgi trafficking (Submission 318)
Jiahe Huang¹, Masaru Fujimoto¹, Masayuki Fujiwara², Yoichiro Fukao², Shin-Ichi Arimura¹, Nobuhiro Tsutsumi¹
¹The University of Tokyo, Japan, ²Nara Institute of Science and Technology, Japan
Dynamin-Related Protein 2 (DRP2) is involved in regulating post-Golgi trafficking in land plants but its exact function is unclear. The Arabidopsis genome has two paralogous genes encoding DRP2 (DRP2A and DRP2B) that have highly similar amino acid sequences. To elucidate their roles, we analyzed their 1) gene expression patterns in various tissues, 2) molecular interaction, 3) intracellular localization, and 4) responses to pharmacological inhibitors. qRT-PCR and GUS assays showed that both genes are expressed ubiquitously but expression is strongest around root apical meristems and vascular bundles. Yeast two hybrid (Y2H), bi-molecular fluorescent complementation (BiFC), and co-immunoprecipitation mass spectrometry (Co-IP MS) analyses revealed that DRP2A and DRP2B interact with each other in vivo. Fluorescently-labeled DRP2A and DRP2B viewed with confocal laser scanning microscopy (CLSM) and variable incidence angle fluorescent microscopy (VIAFM) were almost completely co-localized. Inhibitors of cytoskeletal integrity and membrane traffic affected the size and number of DRP2A and DRP2B foci around the plasma membrane. Organelle markers showed that DRP2A and DRP2B were localized to endocytic vesicle formation sites on the plasma membrane and some post-Golgi organelles such as TGN and endosomes. These results suggest that DRP2A and DRP2B have important roles in endocytosis and function coordinately in a wide range of post-Golgi trafficking in land plants.

Keywords: endocytosis, dynamin, post-Golgi trafficking

Poster #134. Proline residues in the transit peptides play crucial roles at multiple steps during protein import into chloroplasts (Submission 321)
Dong Wook Lee¹, Yun-Joo Yoo¹, Younghyun Kim¹, Hyangju Kang¹, Yong Jik Lee¹, Seung Jin Woo¹, Inhwan Hwang¹
¹POSTECH, Korea, The Republic of
N-terminal transit peptides (TPs) of nuclear-encoded chloroplast proteins are necessary and sufficient for targeting to chloroplasts. A large number of sequence motifs present in the TPs play crucial roles at various steps during protein import into chloroplasts. However, the sequence information encoded by the TP is still not fully
understood. Here, we provide evidence that proline residues found abundantly in the TPs play a crucial role at multiple steps during chloroplast protein import. We introduced one or more proline-to-alanine (P/A) substitution mutations into the N-terminal region (containing 79 amino acid residues) of RbcS (RbcS-nt) and examined them for chloroplast protein import in protoplasts. Of these P/A mutants, those with two or more P/A substitutions were trapped at the envelope membranes and produced high-molecular-weight aggregates. Moreover, the proline residues in RbcS-nt determine the specificity toward chloroplast chaperones in protein import and also affect the sensitivity of precursors to 26S proteasomal degradation in protoplasts. Based on these results, we propose that proline residues in TPs are crucial for translocation through the import channel and also for removal of unimported precursors from the import channel through backtracking if precursors engaged in the import are not efficiently translocated through the import channel.

Keywords: Protein import into chloroplasts, Transit peptide, Protein translocation

Poster #135. A novel type of mitochondrial fission induced by cold treatment depends on DRP3 without ELM1 in Arabidopsis thaliana. (Submission 370) Shin-Ichi Arimura1, Kenta Katayama2, Nobuhiro Tsutsumi1 1The University of Tokyo, Japan Mitochondrial fission is carried out through contraction by a ring of polymerized dynamin-related proteins, DRP3A and DRP3B in Arabidopsis thaliana. Another plant specific factor, ELM1 localizes the DRP3 to mitochondrial fission sites in Arabidopsis. In these drp3 and elm1 mutants, there are longer and fewer mitochondria by defect of mitochondrial fission. However, more than one mitochondrion is still observed in each cell in the elm1 mutant, suggesting that residual mitochondrial fission without ELM1 occurs. A homologue of ELM1 in the Arabidopsis genome, ELM2 was tested to be involved in the residual mitochondrial fission. However, the ELM2 showed very limited affects on mitochondrial morphology and the elm1/elm2 double mutant also still has some mitochondria in each cells. Moreover, we found that an hour cold treatment increased mitochondrial number both in elm1, elm1/elm2 mutants and unexpectedly also in the wild type, but not in the drp3a mutant. In the elm1 mutant, DRP3A:GFP was observed in the cytosol but it relocalized to mitochondrial fission sites during the cold treatment. These results collectively suggest that 1) there is a novel mitochondrial fission without ELM1, and 2) cold temperature induces such mitochondrial fission transiently by unknown mechanism that localizes DRP3A to mitochondrial fission sites without ELM1.

Keywords: mitochondria, organelle, dynamin

Poster #136. EBS7 is a plant-specific component of the highly conserved endoplasmic reticulum-associated degradation machinery in Arabidopsis (Submission 418) Yidan Liu1, Congcong Zhang2, Dinghe Wang2, Wei Su3, Muyang Wang2, Jianming Li1 1University of Michigan, United States, 2Shanghai Center for Plant Stress Biology, China, 3University of Michigan, United States Endoplasmic reticulum-associated degradation (ERAD) is an essential part of an ER-localized protein quality control system for eliminating terminally misfolded proteins. Recent studies demonstrated that the ERAD machinery is highly conserved in yeast, animals, and plants; however, it remains unknown if the plant ERAD system involves plant-specific components. We have genetically identified and molecularly cloned the Arabidopsis EMS-mutagenized bri1 suppressor 7 (EBS7) gene and discovered that EBS7 encodes an ER membrane-localized ERAD component that is highly conserved in land plants. Loss-of-function ebs7 mutations prevent the ERAD of brassinosteroid-insensitive 1-9 (bri1-9) and bri1-5, two ER-retained mutant variants of the cell surface receptor for brassinosteroids (BRs). As a result of the compromised ERAD process, both bri1-9 and bri1-5 are significantly accumulated in the ER and subsequently leak out of the ER to the plasma membrane where the two structurally-defective but biochemically-competent bri1 proteins could partially activate the BR signaling pathway, causing phenotypic suppression of the bri1-9 and bri1-5 mutants. We discovered that EBS7 accumulates under ER stress and its loss-of-function mutations lead to hypersensitivity to ER/salt stresses. Additional experiments that suggesting a likely biochemical function of EBS7 in the Arabidopsis ERAD process will be presented at the meeting.
Keywords: endoplasmic reticulum-associated degradation, ERAD, brassinosteroid receptor, BRI1, ER stress

Poster #137. To loop or not to loop? The role of the microtubule-associated protein End Binding 1b in root responses to mechanical cues (Submission 438)
Vita Lai¹, Shannon Squires¹, Doris Cheng¹, Sherryl Bisgrove¹
¹Simon Fraser University, Canada

Plant roots are able to penetrate through the soil and direct their growth towards locations where conditions are optimal. Mechanical stimulation is one cue that roots continuously monitor and respond to as they develop in the soil and the microtubule-associated protein End Binding 1b (EB1b) is involved in these responses. EB1b is one of three EB1 family members in Arabidopsis. The EB1 family of microtubule-associated proteins is conserved across eukaryotes. They are known as microtubule plus-end tracking proteins, or +TIPs, because they preferentially associate with the growing ends of microtubules. Our goal is to investigate the role of EB1b in root responses to mechanical cues. To address this question, transgenic eb1b mutants that over-express either wild type or GFP tagged versions of EB1b have been generated and root responses to mechanical cues have been analyzed in the transgenic lines. We found that roots of plants overexpressing EB1b responded less to mechanical cues than plants that express lower levels of EB1b. This result suggests that EB1b has an inhibitory effect on root responses to mechanical cues and the amount of repression correlates with the level of EB1b expression. Roots of transgenic eb1b mutants expressing a version of EB1b with GFP attached to the C-terminus displayed the same response to mechanical cues as did eb1b mutants, suggesting that the GFP moiety is interfering with the normal function of EB1b. To assess the relative roles of the N- and C-terminal domains of EB1b in repressing root responses to mechanical cues, we are generating transgenic eb1b mutants carrying EB1b truncations with either domain removed. The responses of these transgenic lines to mechanical cues will be evaluated.

Keywords: End Binding 1, Microtubule-associated protein, Mechanical stimulation, Root growth

Poster #138. Revisiting the role of microtubules in root hairs (Submission 440)
Hae Ryoung Kim¹, Neilab Amiri¹, Attique Chattha¹, Sherryl Bisgrove¹
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Root hairs are long tubular extensions of root epidermal cells. They facilitate the uptake of water and nutrients by roots and help anchor plants more securely in the soil. Root hairs are first initiated at a specific site on hair-forming epidermal cells (trichoblasts). Once a bulge has formed, the hair elongates by a process known as tip growth. Tip growth refers to a mechanism whereby expansion is localized to one region of the cell, the growing tip. It is well-known that roots treated with drugs that depolymerize microtubules have root hairs that continue to elongate but the resulting hairs are crooked rather than straight. This outcome has led to the conclusion that microtubules are needed to maintain the direction of elongation by stabilizing the position of the growth site. To further understand how microtubules accomplish this, we are investigating the role of the microtubule associated protein, End Binding 1b (EB1b), in tip growth. Roots of eb1b mutants have root hairs that are identical to wild type. However, we identified a mutation in another gene that abolishes root hair elongation in the eb1b genetic background. We named this gene NUBBIN because double mutants have severe root hair elongation defects, producing nubbins that are much shorter than wild type. This finding suggests that microtubules, via EB1b, function synergistically with NUBBIN to regulate root hair elongation.

Keywords: Root hair, Tip growth, Microtubule, End Binding 1b, Microtubule-associated protein

Poster #139. Root responses to mechanical cues and the microtubule associated protein END BINDING 1b: Examining the effects of GFP fusions to the C-terminal tail (Submission 441)
Shannon Squires¹, Vita Lai¹, Doris Cheng¹, Sachini Ariyaratne¹, Sherryl Bisgrove¹
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Root systems anchor plants into the soil and serve as primary sites of water and nutrient uptake. To accomplish these functions roots must navigate through a complex, heterogeneous environment, avoiding obstacles that may impede their growth. As they grow through the soil, roots continuously monitor and respond to combinations of
touch and gravity stimulation. The highly conserved microtubule associated protein END BINDING 1 (EB1) modulates root responses to mechanical cues. Plants carrying mutations in EB1b, one of three family members in Arabidopsis, have greater responses to mechanical cues than wild type, indicating that the EB1b protein functions as a repressor of the response. EB1 belongs to a group of proteins known as +TIPs because they preferentially accumulate on growing microtubule ends. While bound to microtubules, EB1 proteins can affect microtubule growth rates and they interact with a diverse array of additional proteins. Here we find that eb1b mutants expressing EB1b-GFP fusions have roots that respond to mechanical cues in a manner equivalent to untransformed eb1b mutants, regardless of expression level. We also find that the fusion proteins are capable of binding to microtubule ends, and that microtubule growth rates are equivalent in transgenic lines expressing different levels of EB1b-GFP. Taken together these observations show that the fusion proteins are not fully functional. They also suggest that microtubule binding by EB1b is not sufficient for normal repression of root responses to mechanical cues. We propose that the function of EB1b depends on its ability to bind to non-tubulin proteins, and that adding GFP to the carboxy terminus may interfere with these interactions.

Keywords: Microtubules, END BINDING 1 b, Mechanical cues, Root growth

Poster #140. The Arabidopsis ERAD pathway does not require the middle-branch mannose trimming of asparagine-linked glycans on misfolded glycoproteins (Submission 449)
Dinghe Wang¹, Qiji Hu¹, Yang Xia², Zhi Hong², Wei Su², Muyang Wang¹, Jianming Li¹
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Endoplasmic reticulum-associated degradation (ERAD) of misfolded proteins is a key process of an ER-localized protein quality control mechanism. Recent studies in yeast and mammalian cells demonstrated that the ER-type alpha1,2 mannosidase (ERMan1)-mediated trimming of the middle branch of the asparagine-linked-glycan (N-glycan) is a required step for ERAD because the N-linked Mannose(Man)8N-acetylgalactosamine(GlcNAc)2 but not Man9GlcNAc2 is the preferred substrate for an outer-branch mannose-trimming reaction, which generates the necessary ERAD N-glycan signal to tag a terminally-misfolded glycoprotein for degradation. Our earlier studies showed that both brassinosteroid-insensitive 1-5 (bri1-5) and bri1-9, two ER-retained mutant variants of the brassinosteroid receptor BR1, are degraded by a highly-conserved ERAD system in Arabidopsis and that their ERAD requires the conserved ERAD N-glycan signal generated by the outer-branch mannose trimming reaction. The Arabidopsis genome encodes 5 homologs of the yeast/mammalian ERMan1, including two Golgi-type alpha1,2 mannosidases (MNS1 and MNS2) and one ERMan1-homolog (MNS3) that were known to function redundantly in removing the terminal alpha1,2 mannose residue from the middle-branch of N-linked glycans. We are using a reverse genetic approach to investigate if the bri1-5/bri1-9-degrading ERAD process involves the middle-branch mannose trimming of N-glycans on the two mutant BR receptors. Consistent with functional redundancy of MNS1-3 in removing the middle-branch alpha1,2-mannose residue, loss-of-function mns3 mutation had little effect on ERAD of bri1-5/bri1-9 or growth defects of the corresponding bri1-5 and bri1-9 mutants. Surprisingly, overexpression of MNS1 or MNS3 or simultaneously eliminating MNS1-3 had no effect on the growth phenotypes of the bri1-5/bri1-9 mutants or ERAD of the two mutant BR receptors. We also found that the degradation of bri1-5/bri1-9 in the mns1 mns2 mns3 triple mutant background could be effectively prevented by kifunensin, a widely-used inhibitor of alpha1,2 mannosidases that remove alpha1,2 mannose residues from either the middle-branch or the two outer-branches of N-glycans. Taken together, our genetic analyses and associated biochemical experiments demonstrated that the bri1-5/bri1-9-degrading ERAD process in Arabidopsis does not require the middle-branch mannose trimming to generate the conserved N-glycan signal.

Keywords: Endoplasmic reticulum-associated degradation, ERAD, brassinosteroid receptor, BRI1, alpha12-mannosidase

Poster #141. THE PLASTID SEC2 SYSTEM PLAYS A ROLE IN CHLOROPLAST BIOGENESIS. (Submission 451)
Donna Fernandez¹, Rajneesh Singhal², Cullen Vens²
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Plastids contain two distinct Sec protein translocation systems, Sec1 and Sec2. The Sec1 system is well
characterized and has been shown to be important for the translocation of proteins across the thylakoid membranes. It includes the membranous components SCY1 and SECE1, as well as a soluble motor SECA1. Mutations in Sec1 components lead to production of albino seedlings. The Sec2 system, which is preferentially localized in the inner envelope, was more recently identified. All plants, as well as green algae, contain genes for Sec2 components, and mutations in these components typically lead to embryo lethality. We have been developing genetic tools to aid in analysis of Sec2 structure and function. In addition to SCY2 and SECA2, we have identified a protein that may provide Sec6 function, which we have designated as SECE2. Analysis of sece2 mutants, which sometimes survive to the seedling stage, reveals major defects in chloroplast development. We have also developed lines with engineered partial loss-of-function scy2 alleles, which show defects in greening. On the other hand, root development appears to be fairly normal, which suggests that Sec2 function is especially critical for chloroplast development. This is consistent with our hypothesis that one of the major roles of the Sec2 system may be to integrate the multi-pass membrane proteins that form the core of the translocase complexes needed for thylakoid biogenesis. Supported by NSF MCB 1158173.

Poster #142. Sequence features that confer the targeting specificity of mitochondrial signal-anchored proteins (Submission 455)
Junho Lee¹, Hong Hanh Nguyen¹, Myounghui Lee¹, Inhwan Hwang¹
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Membrane proteins are involved in various biological processes in the cell. To carry out their roles properly, membrane proteins need to be targeted to the correct subcellular organelles such as chloroplast (CH), mitochondria (MT), endoplasmic reticulum (ER) and so on. Among several types of membrane proteins, signal-anchored (SA) proteins harbor a single transmembrane domain (TMD) at their N-terminus and the TMD plays as the specific targeting signal. Moderate hydrophobicity (HP) and the C-terminal positively-charged region (CPR) flanking to the TMD C-terminally are the necessary features to mediate proper CH and MT targeting of SA proteins after protein synthesis. In plant, however, CH and MT endosymbiotic subcellular organelles exist together and their SA proteins are targeted to each organelle specifically despite having similar targeting signals. This fact raises the possibility that additional features should be inherent on the targeting sequence to confer the selectivity between CH and MT SA proteins. Here, we analyzed targeting sequences of CH and MT SA proteins to find the determining features and define the underlying mechanism by which they are discriminated specifically. By the sequence comparison, it was revealed that MT SA proteins commonly contain additional sequence features in their targeting signals. Mutations of the sequence features caused to change the targeting pathway of MT SA proteins toward CH, proving that they mediate the MT targeting of SA proteins. Similarly, identical sequence features were also found in the targeting sequences of yeast and human MT SA proteins and important for their specific MT targeting as well, suggesting that sequence features and the sorting mechanism of MT SA proteins are well-conserved universally. Based on the results, we propose that specific sequence features of MT SA proteins play a critical role in the sorting process between CH and MT endosymbiotic organelles and they are coordinated elaborately to accomplish the specific MT targeting.

Keywords: chloroplast, mitochondria, signal-anchored protein, membrane protein, targeting signal

Poster #143. EndoQuant: A Tool for Automatic Quantification of Endomembrane Phenotypes (Submission 474)
Nolan Ung¹, Asong Tambo¹, Bir Bhanu¹, Natasha Raikhel¹
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The endomembrane system is a connected network of organelles that cooperatively coordinate the movement of cellular cargoes to deliver them to their proper destination. Classical high throughput screens for genetic discovery are impractical at the cellular level partly due to the immense amount of time and resources required for analysis. EndoQuant is a novel computational tool that allows researchers to quantify fluorescently labeled cellular structures within the boundary of a cell. EndoQuant counts the number of cells and quantifies cellular structures on a per cell basis, creating a biologically relevant metric. Endoquant also allows for the immediate
visualization of relevant features such as average size per cell, area per cell, average intensity per cell and 20 other various features in numerical or graphical forms. Endoquant also allows users to immediately compare one treatment versus another with various graphs complete with standard error analysis allowing for immediate results and the power to discover a phenotype of interest within seconds. The raw data and graphs can then be exported and displayed at will. Endoquant produces consistent reliable results and will greatly reduce analysis time and facilitate the rapid discovery of underlying cellular mechanisms.

Keywords: endomembrane, image analysis, cell biology, computational tool, automation, phenotyping

**Poster #144. The role of CLASP and microtubule in BR signalling in Arabidopsis** (Submission 476)
Yuan Ruan\(^1\), Geoffrey Wasteneys\(^1\)

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Brassinosteroid (BR) is an essential phytohormone for plant growth and development. The microtubule cytoskeleton has been reported to involve in BR-mediated hypocotyl elongation in Arabidopsis. And BR is also known for regulating root morphology but how this is achieved via microtubule and microtubule associated proteins remains unclear. CLASP is a conservative microtubule associated protein which is crucial for cell division and expansion in different organisms. In Arabidopsis, loss of function clasp-1 root displayed reduced sensitivity to exogenous BR, suggesting that CLASP is required for normal BR signalling. The BR plasma membrane receptor BRI1 was identified as a cargo in SNX1-endosomes, which we characterized to interact with CLASP. BRI1 activity was impaired in clasp-1 mutant. The goal of this research is to understand how CLASP is controlled by BR pathway and its feedback function via BRI1 modulation.

Keywords: Microtubule, CLASP, Brassinosteroid, BRI1

**Poster #145. Do cortical microtubules define the cortex domains that affect the velocity of cellulose-synthase-complexes during directional cell expansion?** (Submission 477)
Miki Fujita\(^1\), Adam Mulvihill\(^1\), Geoffrey Wasteneys\(^1\)

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Most plant cells expand diffusely in one direction. In rapidly elongating cells, both microtubules and cellulose microfibrils align perpendicular to the cell growth axis. Dynamic and organized microtubules are essential in directional cell expansion, however, the exact role of microtubules in cellulose biosynthesis is still unclear. Cellulose-synthase-complexes (CSCs) at the plasma membrane displace their positions as they add glucose molecules to produce cellulose chains. Live-cell imaging of fluorescently-tagged CSCs provides us the velocity and trajectories of the enzyme complexes. Once cellulose is produced, multiple cellulose chains could form crystalline structure and crystalline cellulose affect the mechanical properties of cell walls. The mor1-1 mutant is a temperature sensitive mutant that microtubules become less dynamic and disorganized at restrictive temperature, resulted in forming swollen cells. Previous study has shown that trajectories of CSCs become disordered, more CSCs track in the area where no microtubules are present, average velocity of CSCs and crystalline cellulose content are increased in the mor1-1 mutant at restrictive temperature. These results indicate that microtubules could influence the activity of CSCs and the mechanical properties of cellulose through the interaction with the plasma membrane. CSCs in non-microtubule area might move faster than the ones in microtubule domain. To address this question, we use a total internal reflection fluorescence (TIRF) microscopy and variable-angle epifluorescence microscopy (VAEM) to track fluorescently-tagged CSCs at plasma membrane in the area where microtubules are and are not present.

Keywords: cortical microtubules, cellulose-synthase-complexes (CSCs), directional cell expansion, cellulose biosynthesis, live-cell imaging
Development: Vegetative including root and shoot biology

Poster/Talk #146. The execution of developmental programs in all epidermal cell types are restrained by PPI overaccumulation (Submission 16)
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In Arabidopsis, the vacuolar proton-pumping pyrophosphatase (H+-PPase) is highly active in proliferating young tissues. We reported that PPI-overaccumulation inhibits cell division in cotyledonary palisade tissue cells, and triggers compensated cell expansion. We provided robust evidence that the major function of H+-PPase, during seedling development of Arabidopsis, is removal of inhibitory PPI rather than vacuolar acidification. Here, careful examination of pavement cells revealed that the H+-PPase loss-of-function fugu5 mutant exhibited a much simpler cell shapes than the wild type as deduced from Undulation Index values. Also, number of stomata was significantly increased (~1.7-fold per unit area), whereby stomata cells violated once-cell-spacing rule. All fugu5 phenotypic aberrations were rescued by complementation with the yeast cytosolic PPase IPP1. Similar phenotypic analyses in angustifolia (an)-1 and RIC1 ox suggested that AN, RIC1 and AVP1/FUGU5 act on genetically independent pathways. Importantly, pavement cells in mutants with defects in the glyoxylate cycle or gluconeogenesis, showed no phenotypes, suggesting that lowered sucrose production from triacylglycerol seed reserves is not the cause of fugu5 phenotypes. Although all double mutants between the above three key genes showed additive phenotypes in plant gross morphologies, pavement cells, stomata and trichome developmental defects were synergistically enhanced. Particularly, in an-1 fugu5-1 and an-1 RIC1 ox, the pavement cells showed striking 3D-growth phenotype in addition to aberrant branching and helical arms in trichomes. Together, these findings demonstrate that cytosolic PPI impairs all epidermal developmental programs, probably by restraining the proper distribution and/or dynamism of microtubules, a hypothesis that is now under examination.

Keywords: Pyrophosphate, fugu5 mutant, Epidermis

Poster #147. The GLV6/RGF8/CLEL2 signaling peptide is involved in primordium organogenesis. (Submission 39)
Ana Fernandez¹, Andrzej Drozdzecki¹, Kurt Hoogewijs², Valya Vassileva³, Annemieke Madder², Pierre Hilson³, Tom Beeckman¹
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Signaling peptides have been recently recognized as key factors in cell-cell communication in plants. Converging evidence suggests that they are involved in most plant developmental processes. The GLV/RGB/CLEL peptide family has been shown to control gravitropism, meristem maintenance and root hair development. A role for some GLV peptides has also been suggested in lateral root formation based on gene expression patterns and a reduced number of emerged lateral roots in overexpression mutants. However, no detailed characterization of GLV function in this process has been reported yet. We studied the function of GLV6 during LR initiation. GLV6 is expressed in founder cells before the first asymmetric division and overexpression of the gene disturbs proper primordium development. Furthermore, addition of GLV6 peptide to the growing media affects the early events necessary to produce the first asymmetric divisions leading to primordium formation. Our results suggest that GLV6 participates in the control of correct primordium organogenesis.

Keywords: signaling peptides, GOLVEN/root growth factors/CLE-like peptides, lateral roots

Poster #148. The cell-cycle interactome: a source of growth regulators (Submission 43)
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When plants develop, cell proliferation and cell expansion are tightly controlled in order to generate organs with a determinate final size such as leaves. Several studies have demonstrated the importance of the cell proliferation phase for leaf growth, illustrating that cell-cycle regulation is crucial for correct leaf development. A large and complex set of interacting proteins that constitute the cell-cycle interactome controls the transition from one cell-
cycle phase to another. Here, we review the current knowledge on cell-cycle regulators from this interactome affecting final leaf size when their expression is altered, mainly in Arabidopsis. In addition to the description of mutants of CYCLIN-DEPENDENT KINASES (CDKs), CYCLINS (CYCs), and their transcriptional and post-translational regulators, a phenotypic analysis of gain- and loss-of-function mutants for 27 genes encoding proteins that interact with cell-cycle proteins is presented. This compilation of information shows that when cell-cycle-related genes are mis-expressed, leaf growth is often altered and that, seemingly, three main trends appear to be crucial in the regulation of final organ size by cell-cycle-related genes: (i) cellular compensation; (ii) gene dosage; and (iii) correct transition through the G2/M phase by ANAPHASE PROMOTING COMPLEX/CYCLOSOME (APC/C) activation. This meta-analysis shows that the cell-cycle interactome is enriched in leaf growth regulators, and illustrates the potential to identify new leaf growth regulators among putative new cell-cycle regulators. At the moment, we are functionally characterising one of these new leaf growth regulators in more detail.

Keywords: cell cycle, leaf development, interactome

Poster #149. How mutations in ribosomal protein genes affect leaf polarity in the asymmetric leaves 2 genetic background (Submission 45)
Gorou Horiguchi¹, Mikito Inoue¹, Hidenori Masuda¹, Miyuki Nakata², Iwai Ohbayashi³, Munetaka Sugiyama⁴, Hirokazu Tsukaya⁵
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Mutations in a ribosomal protein (r-protein) gene in Arabidopsis thaliana often cause a wide range of developmental defects throughout their life cycle. Even more interestingly, a novel phenotype arises when an r-protein mutation is combined with a second mutation such as asymmetric leaves 2 (as2). Among a number of r-protein mutants we have investigated, rpl4d was found to be one of the strongest enhancers of the leaf polarity defect of as2 and produced needle-like abaxialized leaves. To understand the molecular basis of this phenotype, we focused on two key issues. The first one is the identification of genes whose expression is affected by rpl4d and the second one is the nature of the molecular defect caused by rpl4d that triggers leaf abaxialization.

To identify key genes involved in leaf abaxialization, we carried out suppressor screening using rpl4d as2 and isolated mutants with as2-like flat leaf blade. Most of them were semi-dominant in the rpl4d as2 background. Among them we identified mutations in two NAC-domain transcription factor genes, SUZAKU1 (SZK1) and SUPPRESSOR OF RID TWO 1 (SRIW1). Both genes were upregulated in rpl4d and rpl4d as2. Thus, semi-dominant behavior of these mutations reflects a dose-dependent effect of the functional alleles. These mutants were also able to suppress leaf abaxialization phenotype in double mutants between as2 and other r-protein mutants. Furthermore, szk1 also suppressed the enhanced leaf abaxialization of as2 by a second mutation in a gene encoding a component of the Elongator complex, which functions in tRNA modification, and that of proteasome, but not by mutations in ANGUSTIFOLIA3 and genes involved in tasiRNA production. These results suggest that SZK1 and SRIW1 are upregulated in response to compromised cellular processes mediated by ribosome and proteasome and cause leaf abaxialization.

We are also investigating the nature of molecular defect caused by rpl4d that triggers leaf abaxialization. We noted that rpl4d alleles are not likely to be null because mutant versions of RPL4D mRNA were accumulated at a low level. We overexpressed rpl4d-3 mRNA, which encodes a C-terminally truncated protein, in as2 and found that it enhanced leaf abaxialization. A complementary experiment wherein RPL4D is downregulated by RNAi in the as2 is now in progress to discriminate whether the production of aberrant form of RPL4D or the reduction of functional RPL4D is responsible to leaf abaxialization.

Keywords: as2, rpl4d, ribosome, abaxial/adaxial patterning, NAC domain transcription factor

Poster/Talk #150. Transcriptional regulation of organ growth under limiting conditions (Submission 54)
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Keywords: as2, rpl4d, ribosome, abaxial/adaxial patterning, NAC domain transcription factor
The ability to optimize organ growth to a large variety of environmental conditions is instrumental for plants to survive. Plants tune their growth by restricting the time and position of cell proliferation and expansion. The spatiotemporal position, where cells stop division and start expansion, also called the transition zone, correlates with a large amount of transcriptional changes. However the key transcriptional regulators important for positioning the transition zone are unknown. For the identification of these key growth regulatory transcription factors, we screened for growth defects in a pool of transcription factors dominantly repressed by fusing them to a strong repression domain or ectopically activated by fusing them to a chemical inducible promoter. We identified a set of growth regulatory transcription factors (GRTFs) that showed an altered position of the transition zone. One transcription factor, GRTF7, showed a delayed transition causing ectopic cell division when ectopically activated. Interestingly, the expression of this transcription factor is mainly associated with non-dividing cells and is upregulated when treated with abscisic acid (ABA) or mannitol. The growth of an insertion mutant, grtf7-1, with reduced expression of GRTF7 was more strongly impaired than wild type when treated with ABA. Transcription profiling on gain-of-function and loss-of-function mutants revealed that GRTF7 acts primarily as a transcriptional repressor. We are currently performing genetic studies with candidate targets to reveal how GRTF7 affects the expansion onset and dictates the positioning of the transition zone under limiting conditions.

Keywords: Transcription control, Cell division, Cell expansion, Abscisic acid

**Poster #151. Embryonic and Vegetative TETRASPANIN Gene Activities Identify Functions in Specific Tissues, Domains and Cell Types** (Submission 59)
Feng Wang\(^1\), Mieke Van Lijsebettens\(^1\)
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The Arabidopsis thaliana (Arabidopsis) TETRASPANIN (TET) gene family consists of 17 members that code for conserved membrane proteins of which the function in plant development is poorly understood. Analysis of the expression of TET promoter:GFP-GUS reporter constructs during embryogenesis, in primary and lateral roots (LRs), in the shoot apical meristem (SAM) and rosette leaves, showed mainly distinct activities of the duplicated TET genes, indicating diverged functions in plant development. The early activities in the SAM, vascular tissue and quiescent center progenitor cells during embryogenesis or in precursor cells of specific cell types after germination, suggest a role in cell identity maintenance or cell fate specification respectively, as revealed by the mutational analysis. Our data indicate that TET genes are new components in cell-to-cell communication processes related to tissue, domain or cell specification.

Keywords: TETRASPANIN genes, Expression analysis, Mutational analysis

**Poster #152. Genetic interactions between GL2, HDG11 and MYB23 transcription factors in maintenance of the trichome cell fate** (Submission 74)
Aashima Khosla\(^1\), Janet Paper\(^1\), Allison Boehler\(^1\), Amanda Bradley\(^1\), Titus Neumann\(^1\), Kathrin Schrick\(^1\)
\(^1\)KANSAS STATE UNIVERSITY, United States

The class IV HD-Zip transcription factor GLABRA2 (GL2) is an important player in controlling the differentiation of leaf trichomes in the epidermis of Arabidopsis. We studied a genetic interaction with a related family member, HOMEODOMAIN GLABROUS11 (HDG11), which was previously identified as a negative regulator of trichome branching. In comparison to gl2 single mutants, we demonstrate that gl2 hdg11 double mutants display enhanced trichome cell-type differentiation defects. Trichome-specific expression of HDG11 in gl2 mutants can partially suppress the trichome defects, and vice versa, overexpression of GL2 in hdg11 mutants partially complements the ectopic branching phenotype of hdg11 mutants. Similar enhanced trichome defects in gl2 hdg11 and gl2 myb23 double mutants and the triple mutant led us to explore a connection to the R2R3-MYB transcription factor MYB23, which is thought to act upstream of GL2. Surprisingly, transcript expression of MYB23 was significantly reduced in shoots from gl2 mutants. Yeast one-hybrid, chromatin immunoprecipitation (ChIP), and reporter gene

**Poster #153. Genetic Interactions between GL2, HDG11 and MYB23 Transcription Factors in Maintenance of the Trichome Cell Fate** (Submission 74)
Aashima Khosla\(^1\), Janet Paper\(^1\), Allison Boehler\(^1\), Amanda Bradley\(^1\), Titus Neumann\(^1\), Kathrin Schrick\(^1\)
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**Poster #155. Embryonic and Vegetative TETRASPANIN Gene Activities Identify Functions in Specific Tissues, Domains and Cell Types** (Submission 59)
Feng Wang\(^1\), Mieke Van Lijsebettens\(^1\)
\(^1\)VIB Department of Plant Systems Biology, Belgium

The Arabidopsis thaliana (Arabidopsis) TETRASPANIN (TET) gene family consists of 17 members that code for conserved membrane proteins of which the function in plant development is poorly understood. Analysis of the expression of TET promoter:GFP-GUS reporter constructs during embryogenesis, in primary and lateral roots (LRs), in the shoot apical meristem (SAM) and rosette leaves, showed mainly distinct activities of the duplicated TET genes, indicating diverged functions in plant development. The early activities in the SAM, vascular tissue and quiescent center progenitor cells during embryogenesis or in precursor cells of specific cell types after germination, suggest a role in cell identity maintenance or cell fate specification respectively, as revealed by the mutational analysis. Our data indicate that TET genes are new components in cell-to-cell communication processes related to tissue, domain or cell specification.

Keywords: TETRASPANIN genes, Expression analysis, Mutational analysis
experiments in planta indicate that an L1-box in the MYB23 promoter acts as a GL2 transcription factor-binding site. Taken together, our findings provide evidence for a previously elusive redundancy between two members of the class IV HD-Zip transcription factor family, and unveil a positive feedback loop in the maintenance of GL2 activity during trichome cell-type differentiation via MYB23.

Keywords: Leaf trichomes, Class IV HD-Zip transcription factors, Functional redundancy, MYB23

Poster #153. Defining novel gene regulatory networks in the Arabidopsis root (Submission 85)
Colleen Drapek¹, Philip Benfey¹
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A cell's trajectory from stem cell to differentiation, while often portrayed as a linear progression, is best described as a network that produces a mature state through several pathways acting together. There are few examples that describe gene regulatory network changes during the entire trajectory of cell differentiation. The goal of my project is to define a gene regulatory network that describes differentiation from stem cell to differentiated cell in the Arabidopsis thaliana root. One cell type of the root, the endodermis, is particularly suitable for generating a regulatory network because the molecular components required for its formation and terminal differentiation are established. These components constitute the foundation of a gene regulatory network that describes progression to differentiation. I am combining three approaches to connect stem cell specification to differentiation at the level of gene expression. First, I identified novel regulators of endodermal differentiation using a forward genetics screen and am characterizing their role in root development. Second, using a yeast-one-hybrid approach, I will build a predictive gene regulatory network upstream of these differentiation regulators. Lastly, whole transcriptome analysis of wild-type and mutant endodermal cells will be used to understand how novel regulators directly affect gene expression. Completion of these studies will further our understanding of basic principles of cell fate determination.

Keywords: Stem cell, Differentiation, Root, Gene Regulatory Networks

Poster/Talk #154. Irreversible fate commitment upon exit from stem-cell-like divisions in the Arabidopsis stomatal lineage requires a FAMA and RETINOBLASTOMA-RELATED module (Submission 93)
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Plants exhibit remarkable plasticity in development and their cells are typically considered totipotent, in that a complete plant can be regenerated from isolated individual cells. In intact plants, however, distinct cell lineages emerge and terminal fates are stable. The stomatal lineage is an important example of a specialized lineage established in the Arabidopsis leaf epidermis. In stomatal development, a transient self-renewing (stem cell) phase creates precursors that differentiate into one of two epidermal cell types, guard cells or pavement cells. The basic helix-loop-helix (bHLH) transcription factor FAMA is the master regulator of guard cell identity; it is necessary and sufficient for guard cell fate acquisition and its expression is limited to guard mother cells and young guard cells. We found that FAMA is also required for the irreversible differentiation of guard cells and that it fulfills this role through recruitment of the Arabidopsis RETINOBLASTOMA homologue, RBR. RBR has been shown to regulate cell divisions, but by retaining RBR levels but disrupting the FAMA-dependent activity we uncoupled division and terminal fate modulating roles of RBR. Specifically, point mutations abrogating FAMA-RBR interactions render FAMA capable of promoting initial guard cell identity, but unable to maintain commitment. Instead, young guard cells are reprogrammed into stomatal precursor cells that recapitulate the stomatal developmental pathway and produce guard cells within guard cells. By monitoring gene expression of earlier developmental stages, we can show that this "reset" is not a global reset to totipotency, but to stomatal lineage ground states. Furthermore, we present evidence that the resetting is due to a loss of a specific FAMA activity (RBR recruitment) rather than FAMA gain of function, as has been proposed previously. Through cell-type specific ChIP, we could demonstrate that FAMA and RBR bind to the regulatory regions of stomatal precursor genes; importantly, RBR is no longer recruited to these sites when its interaction with FAMA is disrupted. Our experiments define a molecular mechanism by which the ubiquitously expressed RBR is recruited to specific genomic contexts at specific times to promote silencing of stem cell regulators, likely made permanent by
chromatin modification. These discoveries shed new light on the way iterative divisions and terminal differentiation are coordinately regulated in stem cell lineages.

Keywords: Stomatal development, Terminal differentiation of guard cells, FAMA, RETINOBLASTOMA-RELATED PROTEIN

Poster #155. brassinosteroids control root epidermal cell fate via direct regulation of a MYB-bHLH-WD40 transcriptional complex by GSK3-like kinases in arabidopsis (Submission 94)
Yinwei Cheng1, Wenjiao Zhu1, Yuxiao Chen1, Xuelu Wang2
1Fudan University, China, 2Huazhong Agricultural University, China

The Arabidopsis root epidermal cell types are defined by position in a predictable manner. Hair cells, or trichoblasts, are specified early from root epidermal cells that lie over clefts between two underlying cortical cells, whereas the root epidermal cells that lie over a single cortical cell develop as non-hair cells, or atrichoblasts. Hair cell and non-hair cell files are patterned alternately in rows within the Arabidopsis root epidermis, with columns of hair cells interspersed with columns of non-hair cells. The root hair and non-hair cell fates are determined by a MYB-bHLH-WD40 transcriptional complex in a position predictable manner and are regulated by many internal and environmental cues. brassinosteroids (BRs) play important roles in regulating root hair specification by unknown mechanisms. Here, we systematically examined root hair phenotypes in BR-related mutants, and found that BR signaling inhibits root hair formation through GSK3-like kinases or upstream components. We found that with enhanced bBR signaling, GL2, a cell fate marker for non-hair cells, is ectopically expressed in hair cells, while its expression in non-hair cells is suppressed when BR signaling is reduced. Genetic analysis demonstrated that BR-regulated root epidermal cell patterning is dependent on the WER-GL3/EGL3-TTG1 transcriptional complex. One of the GSK3-like kinases, BIN2, interacted with and phosphorylated EGL3, and EGL3s mutated at phosphorylation sites were retained in hair cell nuclei. BIN2 can also phosphorylate TTG1 to inhibit the activity of the WER-GL3/EGL3-TTG1 complex. Thus, our study explains how BR signaling regulates both the formation and activity of the WER-GL3/EGL3-TTG1 complex through GSK3-like kinases to coordinate root epidermal cell fate specification. Root hairs are major outgrowth of root epidermal cells increase root surface area and account for majority of the absorbed water and mineral nutrients from soil. The increased root hair number in these BR-deficient and perceptional mutants play important role to enhance plant drought tolerance, which will also be presented and discussed. (Cheng et al., eLife 2014; 10.7554/eLife.02525)

Keywords: Root hairs, Brassinosteroids, EGL3, GSK3-like kinases, TTG1, Cell fate determination

Poster/Talk #156. Active growth reduction of leaves under osmotic stress is executed by decreasing SPEECHLESS protein in Arabidopsis (Submission 96)
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Developmental programs ensure robust formation of basic forms of plants. On the other hand, plant growth largely depends on environment. Plant growth is reduced under different stresses, and lines of evidence have suggested that reduction in growth is an active and adaptive response to adverse conditions. An intriguing hypothesis would be that a stress-induced signaling pathway regulates developmental program.
At an early stage of leaf development, a fraction of protodermal cells acquire the meristemoid mother cell (MMC)-identity. The MMC is a proliferative cell, and initiate the stomatal lineage, which eventually produces stomata and pavement cells. The secretory peptide EPF2 is produced by the MMC, and functions as a negative regulator of new MMC formation. Another secretory peptide, Stomagen, is a positive regulator of stomatal formation. These antagonistically-acting ligands are perceived by a putative receptor complex consisting ER-family and TMM proteins, and the signal is transmitted through a MAP-kinase cascade, which phosphorylate the SPCH transcription factor. SPCH is necessary to enter into stomatal lineage pathway, and SPCH is destabilized by phosphorylation. We found that osmotic stress decreases the amount of key transcription factor SPEECHLESS (SPCH) through a MAP Kinase cascade. This results in decrease in MMCs, which eventually decreases leaf cell number under the stress. But upstream regulators, ER-family receptors and TMM were not required for the
reduction in epidermal growth induced by osmotic stress. Upstream EPF-family peptides were also not required for osmotic stress-induced growth changes: upon stress induction, the expression level of EPF1 was unchanged and of EPF2 was decreased. STOMAGEN was repressed by osmotic stress, but STOMAGEN, repression was also not responsible for decrease in cell proliferation.

Our results show that Arabidopsis responds to osmotic stress by reducing the growth of epidermal cells by decreasing leaf stem cell numbers, this response is mediated by MAPK-mediated decrease in SPCH, and is necessary and sufficient to adapt to stress-response. Thus this study demonstrates a way in which plants modify the core developmental program to adapt to stress conditions.

Keywords: SPEECHLESS, osmotic, adaptive

**Poster #157. The SHORT-ROOT Pathway in the Arabidopsis Shoot (Submission 101)**
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The GRAS transcription factor SHORT-ROOT (SHR), mostly together with its direct target SCARECROW (SCR), plays key roles in root development, including ground tissue patterning, stem cell maintenance, and vascular development in Arabidopsis. In addition, it is shown that the two GRAS transcription factors act as positive regulators in proliferative cell divisions in the leaf and that SHR and SCR, along with SCARECROW-LIKE 23 (SCL23), the close homolog of SCR, regulate specification of the leaf bundle sheath (known as equivalent to the endodermis). Compared to what is known in the root, the SHR pathway in the shoot, however, is largely unexplored. Here we demonstrate that SHR acts as a master regulator, directly regulating the expression of SCR and SCL23 in the SHR pathway. Furthermore, we reveal a novel feedback loop, which controls the movement of SHR protein and the expression of SHR mRNA to fine-tune SHR levels for its proper activity/function. Notably, loss of SHR function causes switching from indeterminate to determinate shoot growth, through reduced proliferation and aberrant differentiation in the shoot vascular stem cells. Our genome-wide expression analyses reveal that SHR regulates expression of genes that are likely involved in vascular development. In particular, we found that SHR controls transcription of a cell-wall modifying enzyme gene (xyloglucan endotransglucosylase/hydrolase) by directly binding to its promoter region. Therefore, we propose that the master regulator SHR, similar to its role in the root, controls the growth and development of the Arabidopsis shoot via stem cell maintenance in the vascular cambium.

Keywords: Gene regulatory networks, GRAS, SCARECROW, SCARECROW-LIKE23, Shoot growth, SHORT-ROOT, Vascular stem cell

**Poster #158. PEAPOD2 regulates leaf growth through its binding to the promoter of CYCD3s genes and its interaction with KIX8 and KIX9 (Submission 119)**
Nathalie Gonzalez¹, Laurens Pauwels¹, Liesbeth De Milde¹, Alexandra Baekelandt¹, Astrid Nagels Durand¹, Jelle Van Leene¹, Geert De Jaeger¹, Alain Goossens¹, Dirk Inze¹
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In a fixed environment, the final size of plant organs, such as leaves, is constant, implying that organ growth is tightly controlled by genetic factors. Two effector systems, cell division and cell expansion, contribute to final organ size. After emergence from the shoot apical meristem, the leaf primordium grows mainly through cell proliferation. This phase of growth is progressively replaced, in a distal-proximal manner, by a period of cell expansion associated, in Arabidopsis, with an alternative mode of cell cycle activity, namely, endoreduplication. Although most of the cells start to differentiate during that period, some, called dispersed meristemoids, still undergo several rounds of division and will form specific cell types, the stomatal guard cells.

The Arabidopsis PEAPOD (PPD) proteins are putative transcription factors that regulate leaf development through the inhibition of meristemoid division. They are related to Jasmonate ZIM domain (JAZ) proteins, central regulators in the signaling cascade of the phytohormone jasmonate. Using a combination of transcript profiling and tandem chromatin affinity purification experiments, we determined PPD target genes during leaf development. We found that PPD2 binds to the promoter of several genes among which are two CYCLIN D3 genes. In addition, we characterized PPD2 protein complexes and identified two uncharacterized proteins KIX8
and KIX9 to interact with the PPD-specific PPD domain. In metazoa, the KIX domain in co-activating histone acetyl transferases mediates interaction with transcription factors. We are currently characterizing T-DNA insertion lines in the KIX8 and KIX9 genes at phenotypic and molecular level.

Keywords: leaf size, meristemoid division, transcriptional regulation

Poster #159. The CLAVATA1 receptor kinase controls plant stem cell niche size via transcriptional repression of related receptor kinase family members. (Submission 121)
Zachary Nimchuk1, Yun Zhou2, Paul Tarr3, Brenda Peterson3, Elliot Meyerowitz4
1Virginia Tech, Caltech, HHMI, United States, 2Caltech, United States, 3Virginia Tech, United States, 4Caltech, HHMI, United States
The CLAVATA3 (CLV3)-CLV1 ligand-receptor kinase pair negatively regulate shoot stem cell proliferation in plants. clv1 null mutations are weaker than clv3, but are enhanced by mutations in the related receptor kinases BAM1-3 (Barely Any Meristem1-3). The basis of this genetic redundancy is unknown. Here we demonstrate that the apparent redundancy in the CLV1 clade is, in fact, due in the transcriptional repression of BAM genes by CLV1 signaling. CLV1 signaling in the rib meristem (RM) of the stem cell niche is necessary and sufficient for stem cell regulation. CLV3-CLV1 signaling in the RM specifically represses BAM expression from the RM in wild type plants. In clv1 mutants, ectopic BAM expression in the RM conditionally complements the loss of CLV1. BAM regulation by CLV1 is distinct from regulation of WUSCHEL, a proposed CLV1 target gene. These data provide an explanation for the genetic redundancy seen in the CLV1 clade, and provide novel insights into the role of CLV1 in stem cell regulation.

Keywords: stem cells, receptor kinase, signaling

Poster #160. ERECTA family genes are essential regulators of shoot apical meristem function. (Submission 125)
Elena Shpak1, Ming-Kun Chen1, Klaus Palme2, Franck Anicet Ditengou2
1University of Tennessee, United States, 2Albert-Ludwigs-University of Freiburg, Germany
In Arabidopsis the ERECTA family (ERf) consists of three leucine-rich repeat receptor-like kinases, ERECTA, ERL1, and ERL2. These receptors are localized in the plasma membrane where their activity is regulated by a family of secreted small proteins from the EPF/EPFL family. ERfs signaling pathway regulates many developmental and stress responses including stomata formation and aboveground plant growth. Our recent data have demonstrated that ERfs also contribute to shoot apical meristem (SAM) maintenance, efficient leaf initiation, and establishment of phyllotaxy. The vegetative SAM in er1 er2 mutant is dramatically increased in size and initiates leaf primordia at a reduced rate and with almost random divergence angle. Analysis of DR5rev::GFP expression in er1 er2 suggested that auxin accumulates at a high level in the L1 layer of the SAM, but is not able to form maxima or move into the vasculature. This abnormal auxin distribution is likely due to impaired transport at least in part caused by changes in PIN1 expression. While in er1 er2 PIN1pro:PIN1-GFP was detected in the L1 layer of the SAM, canalization of PIN1 expression to the forming midvein was dramatically decreased in the mutant. Interestingly, the influence of ERfs on auxin transport is specific to PIN1 in the SAM and in incipient leaves: ERfs do not significantly alter PIN2 and PIN3 expression, and while ERfs and PIN1 are co-expressed during embryogenesis ERfs do not regulate PIN1 expression at that developmental stage. Currently we are investigating genetic interactions between ERECTA family genes and PIN1.

Keywords: shoot apical meristem, leaf initiation, auxin transport, ERECTA, PIN1

Poster #161. Direct roles of SPEECHLESS in lineage specification, asymmetric cell division and hormone regulation during stomatal initiation (Submission 126)
On Sun Lau1, Kelli Davies1, Jessica Chang1, Jessika Adrian1, Matthew Rowe1, Catherine Ballenger1, Dominique Bergmann1
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Lineage-specific stem cells are critical for the production and maintenance of specific cell types and tissues in
multicellular organisms. In higher plants, the initiation and proliferation of stomatal stem cells is controlled by the basic helix-loop-helix transcription factor SPEECHLESS (SPCH). The stomatal stem cells and SPCH, which represent an innovation in seed plants, allow flexibility in the production of stomata, but how SPCH generates these stem cells is unclear. Here, we developed a highly sensitive chromatin immunoprecipitation (ChIP) assay and profiled the cell-type specific genome-wide targets of Arabidopsis SPCH in vivo. We found that SPCH directly controls key and novel regulators that drive cell fate and asymmetric cell divisions and enhances responsiveness to cell-cell communication. Our results provide molecular insights on how a master transcription factor generates an adult stem cell lineage that contributes to the success of land plants.

Keywords: Stomatal development, ChIP-Seq, Lineage-specific stem cells

Poster #162. Chemical genetic analyses infer that AS1-AS2 controls cell division through ETTIN in leaf adaxial-abaxial and medio-lateral patterning (Submission 144)
Ayami Nakagawa¹, Hiro Takahashi², Takuma Ito¹, Shoko Kojima¹, Yasunori Machida², Chiyoko Machida¹ ¹Graduate School of Bioscience and Biotechnology, Chubu University, Japan, ²Graduate School of Horticulture, Chiba University, Japan

Leaves develop as flat lateral organs from a shoot apical meristem. Initially, a group of cells is patterned along the proximal-distal axis and then the adaxial-abaxial axis is established, which is crucial for further leaf development. Subsequent cell proliferation along the medial-lateral axis results in flat and medio-lateral symmetric leaves. We reported regulatory mechanisms of the ASYMMETRIC LEAVES1 (AS1)-AS2 complex on adaxial-abaxial polarity that represses the ETTIN (ETT)/AUXIN RESPONSE FACTOR3 (ARF3) directly and indirectly represses both ETT and ARF4 by post-transcriptional gene silencing (PTGS) (Iwasaki et al., 2013). Both ETT and ARF4 function to promote abaxial identity. In addition, many mutations, including those in ribosomal protein genes and genes involved in chromatin modification, have been identified that enhance the adaxial-abaxial polarity defects of as1 and as2 mutants. We have found that the AS1-AS2 repression of ETT is important for leaf adaxial-abaxial and medial-lateral axis formation (Iwasaki et al., 2013; Takahashi et al., 2013). However, the roles of the causative genes (so called modifiers) in leaf development, and mechanisms controlling AS1-AS2 downstream ETT have remained elusive. To reveal the repression mechanisms of ETT by AS1-AS2 and modifiers, we screened a chemical library comprising 2,207 natural compounds. We found that 27 of them inhibited plant growth, and 9 of 27 compounds exhibited leaf abnormalities in as1 and as2 plants. Among them, DNA damage-inducing compounds including camptothecin (CPT, an inhibitor of type IB DNA topoisomerase) inhibited the AS1-AS2-mediated leaf adaxialization. Mutations of TOP1a enhanced the leaf abnormalities of as1 and as2; conversely, the introduction of CPT-resistant TOP1 cDNA suppressed these phenotypes. Bioinformatic analysis predicted such genes would be located downstream of AS1, AS2 and ETT. Furthermore, our molecular genetic analysis has indicated that expression of KIP-RELATED PROTEIN5 (KRP5) (for inhibitors of cyclin-dependent protein kinases) was controlled by AS1-AS2 through ETT functions. Additionally, the krp5 mutation suppressed the formation of abaxialized filamentous leaves in the as2 treated with CPT. Our results suggest that AS1-AS2 represses KRP5 for regulating cell division in the medial-lateral axis, and it might result in formation of flat and symmetric lamina. In turn, AS1-AS2 may protect Arabidopsis thaliana from small compound-induced DNA damage.

Keywords: camptothecin (CPT), chemical genetic screening, leaf polarity, ETTIN/AUXIN RESPONSE FACTOR3 (ETT/ARF3), KIP-RELATED PROTEIN5 (KRP5), ASYMMETRIC LEAVES1 (AS1), ASYMMETRIC LEAVES2 (AS2)

Poster #163. Arabidopsis root miRNA Gene regulatory network supports functional role of transcription factors in vegetative development process (Submission 161)
Young Kyoung Lee¹, Lifang Zhang², Allison Gaudinier³, Christophe Liseron-Monfils², Christos Noutsos², Siobhan M. Brady³, Doreen Ware² ¹Cold spring harbor lab., United States, ²Cold spring harbor lab., United States, ³UC Davis, United States

Transcription factors (TFs) and microRNAs (miRNAs) are critical components of a gene regulatory network (GRN), but little is known about genes that regulate miRNA gene and their target genes. Gene expression is regulated at multiple levels, including transcriptional and post-transcriptional levels. Here, we have initially utilized
a gene centered Y1H screening to better understand GRN and to identify key TFs that control the expression of miRNAs involved in the development and stress. Using the Arabidopsis thaliana root as a model, we have screened the promoter sequences of 108 miRNAs and 65 miRNA targets of protein coding genes with a library containing 952 TFs representing approximately 92% of root expressed TFs. The resulting networks contained 2160 protein-DNA interactions (PDI).

To validate these PDIs in vivo, we performed genetic mutant analysis, tobacco transient assay, and transcriptome profiling including RNA-seq and smRNA analysis. Based on our miRNA-related GRN, several TFs families were found to be highly-connected and one such example was the Zinc Finger Homeobox TF family. While none of single loss of function mutants in the Zinc Finger Homeobox TF family didn't exhibit any visible phenotype, multiple knock down mutants, quadruple, amiRNA lines and two independent repressor lines have altered flower structures and enhanced vegetative branching number in Arabidopsis. Therefore, genetic analyses of these mutants suggested that the network could be more generally applied beyond the root system. Using these mutant analyses, we were able to validate a portion of the miRNA GRN; we were also able to show that similar mechanisms were implied during root vascular tissue and flower development.

We further expanded the GRN through integration of protein-protein interactions (PP) and miRNA-mRNA targets (RR) interactions from public data source. We identified several sub-modules, which represent known miRNA functions as well as putative new regulations in development, environmental stress responses and crosstalk between them. Furthermore, it suggests that core root network contains transcription factor modules with functional roles that could also be expanded to other plant tissues.

This work is supported by USDA ARS 1907-21000-030 and it is gratefully acknowledged.

Keywords: vegetative development, miRNA transcription factor, Gene regulatory network, protein-DNA interaction, branching and flower structure

**Poster #164. Root endodermal differentiation requires an uncharacterized MYB family transcription factor (Submission 167)**
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Cellular differentiation is essential for all tissue and organ function, although the relationship between stem cell specification and differentiation is generally not well understood. The Arabidopsis thaliana root endodermis is a tractable model for studying differentiation, which begins with the asymmetric cell division of the Cortex/Endodermal Initial. This stem cell divides to renew itself and produce a daughter cell destined for differentiation. The transcription factor SHORTROOT (SHR) moves from the vasculature into the daughter cell and promotes expression of another transcription factor, SCARECROW (SCR). Together SHR and SCR specify the endodermal and cortex cell fates. They activate expression of cell cycle regulators, resulting in division of the daughter cell. The progeny of this division proliferate to form the endodermis and cortex cell lineages. Mature endodermis develops a cell wall thickening, known as the Casparian Strip. This barrier prevents diffusion of water and other small molecules into and out of the vasculature. Casparian strip membrane domain proteins (CASP) have been shown to anchor the lignin-polymerizing enzymes such that the Casparian strip is deposited in the correct location. We conducted forward genetic screens that identified an uncharacterized MYB family transcription factor required for endodermal differentiation. We show that expression of this transcription factor is controlled by SHR/SCR and that it regulates expression of multiple CASPs. Our results suggest that this transcription factor acts as an intermediate regulator between the SHR/SCR complex and the CASPs, connecting early endodermal specification events with later differentiation processes.

Keywords: Root development, Differentiation, Casparian Strip, Stem cell specification, Endodermis, SHORTROOT, SCARECROW, CASP
**Poster #165. Epigenetic regulator ASYMMETRIC LEAVES2 protein presented around nucleoli and required for establishment of leaf adaxial cell differentiation consistently forms speckles during mitosis**

(Submission 171)

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Leaf primordia with high division and developmental competencies are generated around the periphery of stem cells at the shoot apex. The ASYMMETRIC LEAVES1 (AS1)-AS2 nuclear-protein complex has the crucial role in adaxial-abaxial polarity specification of leaf primordia. The AS1-AS2 complex directly binds the promoter region of AUXIN RESPONSE FACTOR3/ETTIN (ARF3/ETT) gene, which promotes abaxial identity, and represses the expression of ETT (Iwasaki et al., 2013). The AS1-AS2 complex also indirectly represses both ETT and ARF4 by post-transcriptional gene silencing (PTGS) via the miR390-RDR6-tasiRNA pathway. We have also found that AS1-AS2 maintains the status of gene body DNA methylation of ETT. However, information on mechanisms controlling AS1-AS2 downstream gene ETT has remained elusive. The AS2 gene, expressed in leaf primordia, encodes a plant-specific nuclear protein containing an AS2/LOB domain with cysteine repeats (C-motif). AS2 proteins are present in speckles in and around the nucleoli, and in the nucleoplasm of some leaf epidermal cells, together with AS1. We have used the tobacco cultured cell line BY-2 expressing the AS2-fused yellow fluorescent protein to examine subnuclear localization of AS2 in dividing cells. AS2 mainly localizes to speckles (designated AS2 bodies) in cells undergoing mitosis and distributes in a pairwise manner during the separation of sets of daughter chromosomes. Few interphase cells contain AS2 bodies. Deletion analyses have showed that a short stretch of the AS2 amino-terminal sequence and the C-motif play negative and positive roles, respectively, in localizing AS2 to the bodies. Our results suggest that AS2 bodies function to properly distribute AS2 to daughter cells during cell division in leaf primordia; and this process is controlled at least partially by signals encoded by the AS2 sequence itself (Luo et al., 2012). It might be important to know the role of subnuclear localization of AS2, that presents in speckles around the nucleol in dividing cells. While we found that multifunctional nucleolar protein NUCLEOLIN supports in repression of the ETTIN (ARF3) gene expression regulated by AS1-AS2. We will discuss functions of nucleolar and AS1-AS2 speckles in leaf adaxial-abaxial specification.

Keywords: Nuclear speckles, Nucleolus, Leaf development, Cell division, NUCLEOLIN, ETTIN, adaxial-abaxial polarity, ASYMMETRIC LEAVES1

**Talk #166. Threshold dependent transcriptional discrimination and stem cell homeostasis**

(Submission 172)

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Transcriptional mechanisms that underlie dose-dependent regulation of gene expression in plant development are not understood. In Arabidopsis shoot apical meristems, WUSCHEL (WUS), a stem cell promoting transcription factor, moves from the rib meristem (RM) into the adjacent central zone (CZ) where it represses differentiation promoting transcription factors, as well as activate transcription of its own negative regulator-CLAVATA3 (CLV3) by directly binding the promoters. Through cis-element deletion, manipulations of homodimerization threshold of cis-elements and the WUS protein gradient, we show that WUS binds the same cis-elements to activate CLV3 at lower concentration in the CZ and repress CLV3 transcription at higher concentration in the RM. Biochemical evidence shows that WUS binds same cis-elements as monomer at lower concentrations and binds as dimer at higher concentration suggesting that higher concentration induced WUS dimerization represses CLV3 in the RM. Moreover we find that WUS binds several cis-elements, termed cis regulatory module (CRM), in the CLV3 promoter with different affinities and dimerization thresholds. We provide evidence that the CRM responds to different WUS levels in balancing CLV3 levels in the CZ and in excluding CLV3 from the RM. The concentration dependent transcriptional discrimination provides a mechanistic framework to explain stem cell homeostasis that depends on the regulation of the WUSCHEL gradient which is maintained through intrinsic signals.

Keywords: WUSCHEL, CLAVATA, shoot apical meristem, cis regulatory module
Talk #167. NIN-Like Protein 7 (NLP7) Modulates Border-Like Cell Adhesion to Protect the Columella Root Cap in Arabidopsis (Submission 182)
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Most plants produce border cells at the tip of the roots, where they detach and are thought to modify the environment surrounding the root. Border cells are viable cells that may protect the root by secreting substances that change soil nutrients or alter rhizosphere microbial populations. In most species, border cells are released as isolated cells, but in Arabidopsis, border-like cells (BLC) are released as an entire layer from the columella root cap. How BLC release is regulated is unclear, but is thought to involve cell wall modifying enzymes. We have identified a gene, NIN-Like Protein 7 (NLP7), which prevents the release of Arabidopsis BLCs as individual cells, instead promoting the release of BLCs in intact layers. NLP7 encodes a transcription factor in the RWP-RK family and was first identified for its role in nitrate signaling (Castaings et al. 2009). Here we show that mutations in NLP7 lead to the release of isolated BLCs, alterations in the columella root cap and slow root growth. NLP7 is expressed throughout the columella root cap and pNLP7:NLP7:GFP fusions showed that the protein forms a gradient in the columella with increased levels in BLCs. Microarray analysis demonstrated that NLP7 regulates several genes with roles in cell wall modification, a number of which are enriched in the root cap, including CELLULASE5 (CEL5), which is activated in nlp7 mutants. BLCs in cel5 mutants display a 'sticky' phenotype, in which they are neither released as isolated cells nor as whole layers but instead stick to the root forming a clump at the root tip. Our data suggests that NLP7 modulates cell wall degrading enzymes such as CEL5 to protect the root cap from isolated BLC detachment. This appears to be particularly important in stressful environments. In Arabidopsis, low pH causes an increase in the number of BLCs released as isolated cells. However, compared to wild-type plants, nlp7 mutants release significantly more BLCs as isolated cells at low pH. We propose that the release of BLCs as single layers in Arabidopsis serves a protective function for the root cap in stressful environments, and is modulated by NLP7.

Keywords: Border cells, roots, columella root cap

Poster #168. Plasma membrane aquaporins facilitate lateral and adventitious root emergence (Submission 203)
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Lateral roots (LRs) formed below ground from primary roots and adventitious roots (ARs) initiated from aerial tissue are instrumental for plants to acquire nutrients and water under normal and stress conditions. Their development has been shown to be regulated by hormonal cues, in particular by auxin. In Arabidopsis LR and AR formation is initiated in pericycle cells located deep within the primary root or the hypocotyl, respectively. Thus their emergence requires the primordia to penetrate the overlying tissues. Aquaporins are membrane channels that facilitate water movement across cell membranes. Here, we establish a new link between aquaporin-dependent tissue hydraulicis and auxin-regulated root development in Arabidopsis. Most plasma membrane-located aquaporins (PIPs) are repressed during LR emergence (LRE) and by exogenous auxin treatment. Auxin also reduces root hydraulic conductivity both at the cell and whole-organ levels. Based on the cellular expression pattern during LRE of a major isoform, PIP2;1, and positive and negative impacts of water fluxes and turgor changes in the primordium and surrounding tissue, a mathematical model predicted that both loss-of-function and constitutive, ectopic expression of PIP2;1 would delay LRE. This could be confirmed by the phenotypes of the corresponding plant lines. A systematic survey using PIPpro::GUS fusions identified both LR primordium and/or overlying and underlying tissue-expressed PIP isoforms. Consistent with the model prediction, mutation(s) in single, double or multiple LR primordium-expressed PIP(s) result in delayed LRE with PIP2;1 having the strongest impact. In contrast to the model prediction, LRE is also retarded in mutants of PIPs expressed in overlying and underlying tissues. The total number of LRs is not altered in pip mutants showing delayed LRE. Thus PIP
Aquaporins are important to facilitate LRE, but do not regulate LR initiation. AR formation can be synchronized in Arabidopsis hypocotyls similar to LR development. Preliminary data indicate that PIP aquaporins are also facilitating AR emergence, a feature important for flooding tolerance or vegetative propagation of plants.

Peret et al. (2012) Nature Cell Biology 14, 991-998

Keywords: lateral root development, adventitious roots, root branching, auxin, plasma membrane intrinsic proteins, aquaporins, mathematical modeling

Poster #169. Myrosinase idioblast cell fate and development are regulated by the Arabidopsis transcription factor FAMA and polar auxin transport (Submission 206)
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Brassicaeae species harbor a glucosinolate-myrosinase system that acts as a chemically induced defense against insect predation and microorganisms. In Arabidopsis thaliana, myrosinases, also known as Thioglucoside Glucohydrolases (TGGs i.e. TGG1 and TGG2), accumulate inside the tonoplast of guard cells (GCs), as well as in Idioblasts (Myrosinase Idioblasts, "MI") in both vegetative and reproductive organs. While MI cells have been previously described, the genes responsible for their formation have not yet been defined. Here we report that the basic-Helix-Loop-Helix (bHLH) transcription factor FAMA, which is essential for stomatal development, is also required for conferring the fate of MI cells, a finding consistent with both stomata and MI cells harboring myrosinase as well as the expression of a second guard cell fate marker, E1728. Thus FAMA is required for the expression of the myrosinase genes, TGG1 and TGG2, as well as for the cell types that harbor these genes. Although previous reports held that MI cells are located in part of the phloem, we found that instead they are located just outside the phloem and derive from the ground tissue meristem, not the provascular tissue (procambium). Moreover, we report that MI development is regulated by polar auxin efflux and auxin-related signalling since, e.g., the loss of function of the ARF-GEF GNOM gene, as well as the chemical inhibition of auxin efflux, disrupt MI patterning and morphogenesis. These myrosinase cells (stomata and MI cells), constitute part of a wider system that reduces plant predation, the so-called Mustard Oil Bomb, in which breakage of vacuoles in both myrosinase as well as glucosinolate cells (S Cells) yields a brew toxic to many animals, especially insects. Thus the identification of genes that regulate myrosinase cells might help in developing strategies for engineering crops to mitigate predation.

Keywords: myrosinase, JPMI, cell fate, development, FAMA, auxin

Poster/Talk #170. Arogenate Dehydratase 3 (ADT3) plays a key role in the developmental and metabolic switch from seed-to-seeding (Submission 213)
Katherine Warpeha¹, Danielle Orozco-Nunnely¹, Durreshahwar Muhammad¹, Michael Naldrett², Sophie Alvarez², Alessia Para³
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In most eukaryotes, the ability of G-protein signaling to evoke different cellular events in response to a multitude of disparate signals relies on the diversity of G-protein subunits. Similarly, G protein signaling impacts several aspects of Arabidopsis development but it seems to involve only one Ga subunit, GPA1. This suggests that signaling specificity for plant signal transduction mechanisms may reside at the level of effectors. One of GPA1 effectors, Arogenate Dehydratase 3 (ADT3), mediates GPA1 signaling cascade during seedling development. ADT3 catalyzes the last step of phenylalanine (Phe) biosynthesis, and is expressed during seed maturation and early vegetative development. We observed that adt3 mutant seedlings exhibit similar developmental and morphological defects previously reported for gcr1 and gpa1 mutants. 6-day-old dark-grown adt3 seedlings display significantly wider cotyledons than wild type but fewer pavement cells as a result of increased pavement cell size and fewer fully developed guard cells due to impaired meristemoid cell lineage specification. In addition,
Mesophyll cells possess poorly developed plastids lacking an internal membrane network and adt3 seedlings cannot respond to brief UV stress. Supplying exogenous Phe (but not other aromatic amino acids) restores all the observed adt3 phenotypes. Asparagine or NH3Ac could rescue the cell size and cotyledon size defects, but had no effect on the guard cell lineage of adt3 mutants. Conversely, a sucrose treatment restored the guard cell lineage defect in adt3 mutant, but did not correct any cell size defect. We interpret this result as an indication that Phe might be utilized as either a carbon or nitrogen source by the different cell types residing in the cotyledons.

To uncover the molecular basis of ADT3 function in young seedlings, we have undertaken an exploratory proteomic approach. GO term analysis of the adt3 proteome clearly shows that loss of ADT3 significantly affects the levels of proteins involved in the fundamental cellular, developmental and metabolic processes that are known to mediate early seedling development, thus suggesting that ADT3 and the end product of ADT3 activity, Phe, could directly coordinate the protein network subtending seedling establishment (Para et al., 2014, Submitted). Based on these and past reported data, the GPA1-ADT3 nexus may play a pivotal role in the transformation of the heterotrophic embryo into an autotrophic plant.

Keywords: seedling, auxotrophy, chloroplast development, epidermis development, seed-to-seedling transition

**Poster #171. Differentiation and functional specialization of Arabidopsis root cap by NAC transcription factors** (Submission 235)
Masako Kamiya\(^1\), Shinya Higashio\(^1\), Atsushi Isomoto\(^1\), Miyako Nakanishi\(^1\), Shunsuke Miyashima\(^1\), Keiji Nakajima\(^1\)
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Plant root tips are covered with the root cap, a specialized tissue playing important roles, such as protection of the root meristem, perception of gravity and secretion of mucilage to the soil environment. In Arabidopsis, the root cap is composed of a few cell layers of columella and lateral root cap cells, and their differentiation process exhibits unique behavior in that they differentiate from the stem cells with distinct cell lineages, mature rapidly, and finally detach from the outermost layers. The NAC domain transcription factors (TFs), SOMBRERO(SMB), BEARSKIN(BRN)1, and BRN2, have been reported as key regulators of the root cap maturation and detachment. We have identified genes acting downstream of the NAC TFs and possibly function in the root cap maturation/detachment. Most genes were found to encode putative enzymes involved in cell wall or lipid metabolism. Reporter analysis revealed that most genes are expressed specifically or preferentially in limited regions in the outermost root cap layers. Our data suggests the existence of a genetic pathway that controls the root cap maturation and detachment in both spatially and temporally regulated manner.

Keywords: Root cap, SOMBRERO, BEARSKIN

**Poster #172. New stories from an old messenger: different roles of cyclic GMP and nitric oxide during plant development of Arabidopsis thaliana** (Submission 245)
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In mammals, the second messenger cyclic GMP (cGMP) is synthesized by membrane-bound or soluble guanylate cyclases (GCs). The membrane-bound particulate GC (pGC) is activated by natriuretic peptides, whereas the soluble GC (sGC) is stimulated by the diatomic free radical gas nitric oxide (NO). After the activation, GCs catalyze the dephosphorylation of guanosine-5’-triphosphate (GTP) to cGMP. Downstream, cGMP can act on cyclic nucleotide gated channels, protein kinases and/or phosphodiesterases.

In plants, the particulate as well as the soluble form of GCs are also identified. Compared to mammals, where cGMP and NO play an important role during relaxation of vascular smooth muscle cells, in plants cGMP is involved in many different physiological processes, for example in the biotic stress response, during root development, germination and guard cell signaling. Nevertheless, our understanding of cGMP signaling in plants is still poor. Especially the downstream targets of cGMP are unclear.

One major challenge is the determination of cGMP in plants. We used an ELISA-based immunoassay and different treatments to set up a stable method for cGMP measurements in plant tissue. Moreover, we present
evidences for the interplay of cGMP and NO together with the phytohormone auxin during primary root growth and lateral root development. A detailed phenotypically analysis of different mutants combined with the determination of cGMP and NO contents, we identified new genes involved into the cGMP signaling during root development. In addition, using a previously published search motif we identified another putative GC, and we are currently characterizing the corresponding mutants phenotypically concerning cGMP content and root development.

Keywords: cyclic GMP, Nitric oxide, auxin, root development, primary root growth, lateral root growth

Poster #173. Disruption of PME activity alters hormone homeostasis and triggers compensatory mechanisms controlling adventitious rooting (Submission 248)
Steanie Guenin1, Gaelle Mongelard1, Herve Demailly1, Ondrej Novak2, Kieran Lee3, Sophie Bouton1, Petra Amakorova2, Miroslav Strnad2, Paul Knox3, Gregory Mouille4, Jerome Pelloux1, Laurent Gutierrez1
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We recently showed that the decreased PME activity in Arabidopsis pme3 mutant, in which the PECTIN METHYLESTERASE 3 gene was knocked-out, led to increase the degree of methylesterification (DM) of pectins as well as the adventitious root formation in hypocotyl [1]. In order to gain insights into the relationship between DM of pectins and adventitious rooting, we analyzed the Arabidopsis pme36 knock-out line. In wild-type, PME36 is expressed during seed maturation and in seedling hypocotyl up to 72-hours after germination. As expected, pme36 displayed a decrease in PME activity during seed maturation and a resulting increase in the DM of pectins in the mature seed. Unexpectedly, 48-hours after germination, PME activity in dark-grown hypocotyl of pme36 was higher than in the wild-type. This lead to a decrease in the DM of pectins and in the number of adventitious roots in the mutant. While confirming the positive correlation between DM of pectins and adventitious rooting, these results highlight the existence of a mechanism overcompensating the absence of PME36 in pme36 hypocotyl. We found that this compensatory mechanism involved transcriptional regulations, other PME genes being overexpressed in pme36 hypocotyl. In addition, looking at hormone contents and assessing the expression of genes involved in the hormone signaling pathways shown to control adventitious rooting [2], we found a strong alteration of hormone homeostasis in pme36 hypocotyl. Altogether these results suggest that adventitious rooting is controlled by a regulatory network involving crosstalk between hormone signaling and PME activity, which is modulated through a compensatory mechanism triggered by variations in DM of pectins.

Keywords: Adventitious rooting, hormone signaling, pectin methylesterification, transcriptional compensation

Poster/Talk #174. Natural variation of an A-type ARR quantitatively tunes cytokinin dependent cell size determination in the root (Submission 254)
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Shape and sizes of organs are critical for their physiological function. In plants, length, diameter and topology of roots determine the ability to penetrate and explore the soil, acquire nutrients and anchor the plant. Ultimately, these root characteristics are determined at the cellular level and are the consequence of complex regulatory interactions such as those in the auxin and cytokinin signalling pathways. We explored natural phenotypic variation of root growth among 163 Arabidopsis thaliana accessions to dissect the fine-tuning of quantitative growth regulation in plants. Notably, genome wide association mapping for the variation of root diameter identified a genomic region in the regulatory region of an A-type Arabidopsis response regulator (A-type ARR) gene family member, implicated in the negative regulation of cytokinin signaling. The loss of function mutant of this gene showed a significantly increased root diameter when compared to wild type, strongly suggesting that the candidate gene is negative regulator of root diameter. Furthermore, while exogenous cytokinin addition increased root diameter in wildtype and mutant plants, this effect was largely abolished by overexpression of the A-type ARR. Taken together, this strongly suggests that the candidate A-type ARR tunes root diameter by negatively
regulating cytokinin signaling. Moreover, this cytokinin signaling dependent regulation of root diameter is due to regulation of cell sizes. Overall, our findings uncovered a novel molecular link between cytokinin signaling, the natural alleles of an A-type ARR gene and the determination of root diameter, highlighting the role of cytokinin signaling in the quantitative regulation of radial root growth.

Keywords: cell size determination, radial root growth, natural variation

**Poster #175. Identification of factors acting downstream of PXY signalling** (Submission 260)
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Plant vascular tissue is essential for transport of water and nutrients and is constituted of two conducting tissues, the xylem and phloem. Xylem and phloem are derived from pluripotent cells present in the cambium and procambium which are the vascular meristems. The high level of organization in the vascular tissue makes it an interesting model for studying cell division and differentiation. Consequently an emerging network of signaling components and transcription factors has been identified. TDIF, a peptide ligand is encoded by CLE41, CLE42 and CLE44 genes, and has been shown to bind to PXY, a receptor like kinase that promotes properly orientated vascular cell divisions and represses vascular differentiation. In order to identify novel PXY signaling components, the transcriptome of wild type and plants overexpressing CLE41 were compared. Putative transcription factor targets of PXY signaling were identified, and we present genetic evidence demonstrating that they act downstream of the PXY receptor kinase to control vascular development.

Keywords: vascular, signaling, phloem, xylem, cell division

**Poster #176. Trans-acting siRNA-mediated silencing of CER3 controls cuticular wax biosynthesis during Arabidopsis inflorescence stem development** (Submission 263)
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The primary aerial surfaces of land plants are covered by a protective hydrophobic layer called the cuticle, which mediates plant interactions with the environment and prevents organ fusion during development. The cuticle is synthesized by epidermal cells and is composed of the cutin polyester matrix and cuticular waxes. Previously, we uncovered a novel mechanism of regulating cuticular wax biosynthesis during Arabidopsis stem elongation that involves the ECERIFERUM7 (CER7) ribonuclease, a core subunit of the exosome. Loss-of-function mutations in the CER7 gene result in reduced expression of CER3, a key wax biosynthetic gene. We therefore proposed that CER7 ribonuclease degrades an mRNA encoding a repressor of CER3. To identify this putative repressor, we performed a cer7 suppressor screen that resulted in the isolation of components involved in post-transcriptional gene silencing (PTGS), RNA-DEPENDENT RNA POLYMERASE1 (RDR1) and SUPPRESSOR OF GENE SILENCING3 (SGS3), demonstrating that CER3 expression is controlled by small RNAs. To reveal the molecular identity of the effector RNA species, and to determine whether these effector RNAs control CER3 transcript levels directly or indirectly, we cloned and characterized additional genes identified in our suppressor screen and performed next generation sequencing (RNA-seq) of small RNA populations that differentially accumulate in the cer7 mutant in comparison to wild type and cer7rdr1 and cer7sgs3 double mutant lines. We will present our current understanding of the role of these genes and the mechanism of the CER7-dependent regulation of wax biosynthesis in Arabidopsis.

Keywords: regulation of wax biosynthesis, tasiRNA, Arabidopsis

**Poster #177. SPF is required for sieve plate and sieve pore development to mediate long distance cell-to-cell communication** (Submission 265)
Robertas Ursache¹, Jan Dettmer², Ana Campilho³, Shunsuke Miyashima⁴, Ilya Belevich¹, Seana O’regan², Daniel Leroy Mullendore³, Thomas Moritz⁴, Michael Knoblauch⁵, Eija Jokitalo¹, Yka Helariutta¹
In plants, phloem is the tissue that carries photosynthetic products, hormones, proteins and miRNAs throughout the plant body. Primary phloem consists of conducting sieve elements (SE) and neighboring companion cells (CC). At the interface between individual SE are the sieve plate areas with multiple perforations, sieve pores, which facilitate the transport of various macromolecules. Each sieve pore represents a plasma membrane-lined channel, in the centre of which an appressed form of the ER, desmotubule, is found. The space between the plasma membrane and the desmotubule is defined as symplastic space which serves as a route for cell-to-cell communication. During maturation, the sieve elements undergo a selective breakdown and all the remaining components become distributed along the wall. This process is accompanied by plasmodesmatal enlargement and disintegration of the desmotubules which leads to the formation of mature sieve pores. To date, little is known about the molecular framework related to the biogenesis of the sieve areas and sieve pores. In our work we identified mutations in SPF (Sieve Pore Formation) gene of Arabidopsis thaliana causing several phenotypic abnormalities, including impaired phloem transport and disrupted phloem continuity. Furthermore, using serial block-face scanning electron microscopy (SBEM) we observed a reduction in sieve pore density and abnormal sieve pore formation in spf mutant. We show that SPF facilitates the transport of phospholipid precursors; localizes to the trans-Golgi network; and during cell plate formation is associated with the phragmoplast. Consistent with its function in the sieve plate and sieve pore formation, SPF shows a sustained, polar localization in the forming sieve plates. Moreover, we identified a suppressor mutation that significantly restored the phenotype of spf. The identification and further characterization of the suppressor mutation will contribute to a better understanding of the role of SPF in sieve plate and sieve pore formation.

Keywords: Phloem sieve elements, Sieve plate and sieve pore formation, Long distance cell-to-cell communication

Poster #178. The LFM1 gene Controls Leaf Morphogenesis in Arabidopsis (Submission 274)
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Leaf morphogenesis is controlled by the coordination of cell division, cell differentiation and cell expansion. However, the molecular mechanisms underpinning leaf formation are still largely unknown. Here, we demonstrate that LFM1 gene plays important roles in leaf morphogenesis. Overexpression of LFM1 caused deeply serrated. LFM1 gene encodes a putative transcription factor belonging to a superfamily. The LFM1 is localized in nucleus, as indicated by the analysis of the transgenic plants expressing LFM1-GFP fusion protein. The LFM1 gene was expressed in young leaves, with the highest expression in vascular tissues and serration areas. Dominant-negative disruption of LFM1 by expressing LFM1-SRDX driven by its own promoter led to small and round leaves without serration. These results indicates that LFM1 is a transcription factor controlling leaf serration and leaf development.

Keywords: Leaf morphogenesis, LFM1, Transcription factor, Leaf serration

Poster #179. Interaction of cytokinin with auxin to control cell elongation and regulate root growth (Submission 277)
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Cytokinin inhibits root growth through effects on both cell division and cell elongation. Cytokinin signaling operates via a two-component signaling pathway, culminating in activation of the type-B response regulator transcription factors, ARR1, ARR10, and ARR12 being the major contributors to root cytokinin responses in Arabidopsis. We identified the auxin importer AUX1 as a positive regulator of cytokinin responses in the root through a genetic screen. Mutants of AUX1 affect cytokinin-mediated inhibition of root cell elongation but not of root cell division, suggesting that the primary role of the shootward directed auxin transport mediated by AUX1 is in the control of cell elongation. We identified two mechanisms by which AUX1 positively contributes to the cytokinin response.
First, expression of the type-B response regulator ARR10 is reduced in the aux1 mutant, an effect predicted to reduce the sensitivity of the root to cytokinin. Consistent with this prediction, transcript levels of cytokinin primary response genes are also reduced in the aux1 mutant. Second, making use of the DR5:GFP auxin reporter, we found that the auxin activity induced in the root epidermal layer by cytokinin is lost in type-B ARR and aux1 mutants, consistent with a shared role in mediating cytokinin-dependent changes in auxin distribution. Our data support a model in which cytokinin inhibits root elongation through AUX1-dependent polar auxin transport.

Keywords: cytokinin, auxin, root development, type-B ARR, AUX1

Poster #180. Overexpression of Tap46, the PP2A regulatory subunit, leads to enhanced plant growth through stimulation of TOR signaling pathway. (Submission 285)
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Tap46 (Type 2A-phosphatase-associated protein 46kD), plant homolog of Tap42 in yeast and alpha4 in mammals, is one of the major regulators of the TOR pathway by which the gene regulates protein phosphatase 2A (PP2A). We have generated Arabidopsis plants constitutively overexpressing Tap46. The constitutive overexpression line (OE) shows increased growth rate, mainly by cell expansion, along with increased silique size and seed size as well as increased seed viability. Dexamethasone-inducible Tap46 overexpression line (DOE) also shows increased growth rate analogous to that of the OE plants. Nitrogen assimilation activity, which is reduced in Tap46-RNAi lines, is increased in DOE plants. However, glutamine synthetase, involved in nitrogen assimilation as well as nitrogen recycling, is slightly decreased in DOE plants. When Tap46 is overexpressed, S6K phosphorylation is also increased, whereas silencing Tap46 leads to dephosphorylation of S6K1. Interaction of Tap46 with S6K1 and S6K2 was checked. Under conditions of TOR kinase silencing, Tap46 is decreased while PP2Ac is increased, implying the opposing roles of Tap46 and PP2Ac in the TOR pathway. Various genes related to nitrogen metabolism, ribosome biogenesis, lignin biosynthesis, cell wall biogenesis, cytochrome 450, autophagy, and lipid degradation were modulated in DOE lines. This study shows the phenotypic effects of Tap46 overexpression and also elucidates the cause of such phenotypic modulation to the induction of the TOR pathway in Arabidopsis.

Keywords: Tap46, S6K, TOR pathway, plant growth

Poster #181. Identification of a new acetyl-CoA carboxylase 1 mutant allele responsible for the repression of seed storage proteins (Submission 290)
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In plant, seed storage proteins (SSPs) are specifically synthesised in seed maturation stage and are mobilized to provide nutrition during seed germination and early seedling growth. Genes encoding SSPs are highly expressed in maturation stage and repressed in vegetative tissues. However, the mechanisms that repress the expression of these genes in vegetative organs are not well understood. Here, we isolated an ethylmethane sulphonate (EMS)-induced mutant which shows defective cotyledons, short roots and aberrant leaf shape in MS medium. 21 days after germination, about 10% of mutants can develop somatic embryo-like structures on leaves and those structures can be stained by neutral lipid dye fat red. Further, an increased expression of FUSCA3 (FUS3), an activator of seed maturation, was detected in the mutant. Next-generation mapping identified a transition mutation G6164A at the locus encoding homodimeric acetyl-CoA carboxylase 1 (ACC1), which causes single amino acid substitution from aspartic acid to asparagine. Allelism and complementation tests confirm that the mutation of ACC1 is responsible for the mutant phenotypes. In cytosol, homodimeric ACC1 converts acetyl-CoA to malonyl-CoA which serves as the first and rate-limiting reaction in elongation of fatty acids. Exogenous malnoate or mixture of very-long-chain fatty acids can partially rescue the root length of acc1 mutant but not aberrant leaf, somatic embryo like-structures and elevated expression of FUS3. This suggests that other mechanisms rather than the defect of synthesis of very-long-chain fatty acids are responsible for the repression of SSPs in vegetative tissues.
Poster #182. Arabidopsis guard cell integrity involves the epigenetic stabilization of the FLP and FAMA transcription factor genes (Submission 348)
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Arabidopsis guard cell (GC) fate is conferred via a transient pulse of expression of FAMA that encodes a bHLH transcription factor. Stomata often function for years, suggesting that the FAMA expression window stabilizes long-term GC identity or that additional factors operate. Transgenic lines harboring a copy of a FAMA transgene were found to induce the fate resetting of mature GCs to that of lineage-specific stem cells causing new stomata to arise within shells of the old, a Stoma-in-Stoma (SIS) phenotype. These lines disrupt the normal trimethylation on lysine 27 of histone3 (H3K27me3) on stomatal stem cell genes, a phenotype rescued by constitutive expression of the Polycomb Group (PcG) gene CURLY LEAF. Thus the stability of stomatal fate is enforced by a PcG-mediated reduction in the transcriptional accessibility of stem cell genes and by the endogenous FAMA gene itself. Moreover, a transgenic FOUR LIPS gene, which encodes a MYB protein that is not required for GC fate, also induces a SIS phenotype and disrupts H3K27 trimethylation. Thus FLP might indirectly enforce GC fate as well.

Keywords: guard cell, FAMA, FOUR LIPS, cell fate, stomatal development, Arabidopsis

Poster #183. Deep functional redundancy between FAMA and FOUR LIPS in stomatal development (Submission 349)
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Functional redundancy arises between gene paralogs as well as non-homologous genes that play a common role at a shared node. The bHLH transcription factor FAMA, along with the paralogous MYB genes, FOUR LIPS (FLP) and MYB88 all ensure that Arabidopsis stomata contain just two guard cells (GCs) by enforcing a single symmetric precursor cell division before stomatal maturity. Consistent with this function, FLP and FAMA exhibit the same expression pattern in which both translational GFP fusions emit fluorescence just before and after symmetric division; however, FAMA but not FLP is required to confer GC fate. Strikingly, swapping the genes and promoters of the FLP and FAMA genes results in the reciprocal complementation of respective loss-of-function mutants. Thus, an FLP transgene can restore GC fate to a fama mutant background. FAMA, FLP and the FLP paralog MYB88 were previously shown to influence higher order functions in stomatal development, including maintaining and stabilizing stomatal fate. Here we show that these overlapping functions are likely to also involve interactions between FLP and FAMA with the RETINOBLASTOMA-RELATED (RBR) protein.

Keywords: guard cell, FAMA, FOUR LIPS, RETINOBLASTOMA-RELATED, Arabidopsis

Poster #184. Functional characterization of LATERAL ORGAN BOUNDARIES DOMAIN (LBD)13 and LBD14 in Arabidopsis (Submission 366)
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¹Department of Bioenergy Science and Technology, Chonnam National University, Korea, The Republic of The LATERAL ORGAN BOUNDARIES DOMAIN/ASYMMETRIC LEAVES2-LIKE (LBD/ASL) genes encode a plant-specific family of transcriptional regulators harboring a conserved amino acid domain, referred to as the LOB (for lateral organ boundaries) domain. The LOB domain is approximately 100 amino acids in length and contains a conserved four-Cys motif, a Gly-Ala-Ser block and a Leu-zipper-like coiled-coil motif. In Arabidopsis, the LBD gene family comprises 42 members, and are assigned into two classes based on the conserved motifs and amino acid sequence similarity. Class I comprises 36 genes and class II comprises 6 genes. A variety of studies have revealed the roles of certain LBD genes in the development of the leaf, the embryo, tracheary
The plant meristem is a pivotal tissue where cell production takes place to sustain organ growth and organogenesis. The shoot apical meristem (SAM) and root apical meristem (RAM) are well-studied meristems and are located at the tips of the aerial part and root, respectively. Cell production activity in the meristems should be appropriately regulated. To achieve this, organisms have developed scrupulously designed cell-to-cell communication systems during the evolutionary history. In plants, leucine-rich repeat receptor (LRR)-containing receptors play important roles in intercellular signaling, and some are involved in meristem maintenance. The evolutionarily conserved leucine-rich repeat receptor-like kinases (LRR-RLKs) and CLAVATA3 (CLV3) EMBRYO SURROUNDING REGION (CLE) peptide-mediated signaling systems are known to be responsible for setting the cell production activity at appropriate levels, in particular, the CLV pathway and WUSCHEL (WUS) compose a feedback-regulating module in the SAM.

On the other hand, heterotrimeric G proteins, consist of alpha, beta and gamma subunits, are important signaling molecules that link extracellular signals with intracellular mechanisms. The components of the G proteins were conserved in eukaryotes, and plant G proteins are known to be involved in various aspects of morphological and physiological processes. Recently, Bommert et al. (2013, NATURE) reported a genetic evidence that maize G[?] modulate CLV signaling on the control of shoot meristem size. However, the molecular insight of the crosstalk between CLV pathway and G proteins are yet unclear.

Here, we show that the Arabidopsis G protein beta-subunit1 (AGB1) are function synergistically with the CLV signaling pathway. agb1 mutant exhibits an enlarged stem cell region, which is similar to that of clavata mutants. Our genetic and biochemical analyses suggests that RECEPTOR LIKE PROTEIN2 (RPK2), one of three major CLAVATA3 (CLV3) receptors, is responsible for the signal transmission from the CLV pathway to the G protein signaling. Based on the results, we hypothesize that AGB1 is involved in meristem development in the RPK2 receptor-dependent signaling pathway and imply diversity of CLV signaling through G proteins in plants.

Keywords: Heterotrimeric G proteins, CLAVATA signaling, CLE peptide, meristem development

Poster #185. Heterotrimeric G proteins control stem cell proliferation through CLAVATA signaling in Arabidopsis. (Submission 386)
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1Kumamoto University, Japan, 2Duke University, United States

The plant meristem is a pivotal tissue where cell production takes place to sustain organ growth and function. The shoot apical meristem (SAM) and root apical meristem (RAM) are well-studied meristems and are located at the tips of the aerial part and root, respectively. Cell production activity in the meristems should be appropriately regulated. To achieve that, organisms have developed scrupulously designed cell-to-cell communication systems during the evolutionary history. In plants, leucine-rich repeat receptor (LRR)-containing receptors play important roles in intercellular signaling, and some are involved in meristem maintenance. The evolutionarily conserved leucine-rich repeat receptor-like kinases (LRR-RLKs) and CLAVATA3 (CLV3) EMBRYO SURROUNDING REGION (CLE) peptide-mediated signaling systems are known to be responsible for setting the cell production activity at appropriate levels, in particular, the CLV pathway and WUSCHEL (WUS) compose a feedback-regulating module in the SAM.

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Keywords: Heterotrimeric G proteins, CLAVATA signaling, CLE peptide, meristem development

Poster #186. Analysis of CLE peptide signaling in root apical meristem (Submission 387)
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1Kumamoto University, Japan, 2Duke University, United States

The plant meristem is a pivotal tissue where cell production takes place to sustain organ growth and...
organogenesis. The shoot apical meristem (SAM) and root apical meristem (RAM) are well-studied meristems and are located at the tips of the aerial part and root, respectively. Although the structures of the SAM and RAM are different, similar basic mechanisms that balance cell proliferation and differentiation seem to be employed. Ligand-receptor systems are the indispensable molecular basis to perceive extracellular signals for cells. In plants, leucine rich repeat (LRR)-containing receptors play important roles in intercellular signaling, and some are involved in meristem maintenance. The evolutionarily conserved CLAVATA3 (CLV3) /EMBRYO SURROUNDING REGION (CLE) peptide and their receptor-mediated signaling systems are known to be responsible for setting the cell production activity at appropriate levels, in particular, the CLV pathway and WUSCHEK (WUS) compose a feedback-regulating module in the SAM. Despite the clear feedback modules that consist of the positive and negative regulators function in the SAM, it is still unclear whether there are equivalent regulatory mechanisms in the RAM. Recent genome-wide analysis have identified that the various CLE family small peptides are expressed in various tissues. Based on the fact that the CLE peptide inhibits RAM activity and several proteins in the CLAVATA pathway are considered to be involved in this regulation, the presence of a CLV-like signaling pathway in the root meristem was proposed. Here, we conducted a genetic screen to discover components of the signaling pathway. From EMS-mutagenized seed populations, we have isolated 26 mutants that resist to the exogenously applied CLE peptide. Among the mutants, we have determined that BARELY ANY MERISTEM 1 (BAM1), which is a member of the CLV1 class LRR-RLK, is involved in the CLE peptide mediated root growth inhibition. Our genetic analysis revealed that BAM1 have a role in signal transduction together with other LRR-RLK receptors, and the LRR-RLKs constitute signaling pathway that modulates activity of cell proliferation in the root meristem. We also identified genes for the CLE peptide resistance from the mutants. Elucidating the function of the genes will shed light on the molecular basis that is controlling the meristem activities in the RAM.

Keywords: root apical meristem, CLE, BAM1

Poster #187. The Leucine-Rich Repeat Receptor-Like Kinase MUSTACHES Regulates Bilateral Symmetry in Stomata (Submission 393)
Sandra Keerthisinghe¹, Fred Sack¹
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Stomata regulate shoot CO2 uptake in photosynthesis as well as transpirational cooling.Dicot stomata consist of two bilaterally symmetrical and kidney-shaped guard cells (GCs) that enclose an epidermal pore, a symmetry central to stomatal function. This symmetry extends to radially-arranged microtubules that primarily originate at the pore and then elongate outwards.
Mutations in MUSTACHES (MUS), which encodes an LRR Receptor-Like Kinase, disrupt this bilateral symmetry as shown by defects ranging from skewed pores, to paired guard cells that face away from each other, or to a severe loss of stomatal shape. Mutants in mus also display defects in the organization of GC microtubule arrays, in the polarity of microtubule growth, as well as in the localization of components of the MicroTubule Organizing Center (MTOC). Since defects in microtubule organization and movement occur before cell morphogenesis defects can be detected, MUS presumably enforces stomatal symmetry by orchestrating microtubule distribution. MUS translational-GFP fusions exhibit expression in cell plates in most cells types in roots and shoots. However, in recently divided GCs, MUS expression is notably absent from new cell plates, and instead is peripherally located. These results indicate that MUS enforces wall and cytoskeletal polarity at the center of the developing stoma via signaling from the vicinity of the GC membrane.

Keywords: Stomata, Bilateral Symmetry, Morphogenesis, Cytoskeleton

Poster #188. A ROS responsible TF regulates root growth by directly controlling expression of novel protein that modulates cell length. (Submission 400)
Kaho Mabuchi¹, Takamasa Suzuki², Mika Nomoto³, Yasuomi Tada³, Sumie Ishiguro⁴, Wolfgang Busch⁵, Tetsuya Higashiyama⁶, Philip Benfey⁷, Hironaka Tsukagoshi⁸
Homeostasis of reactive oxygen species (ROS) that is regulated by a transcription factor (TF), UPB1, is one of the key components for controlling the plant root growth. However, little is known of how ROS regulates root growth besides UPB1. To elucidate the ROS roles for root growth, we performed time course microarray analysis by using Arabidopsis root treated with H2O2. We have found around 200 genes which showed clear response to H2O2. Among them, we focused on one TF that was named ROS First Response TF1 (RFRT1).

Although RFRT1 expressed very weak in the basal meristem under the control condition, RFRT1 expression was strongly induced by H2O2 and this expression domain expanded from the basal meristem to the elongation zone. Root growth of T-DNA insertion line of RFRT1 (rfrt1-1) mutant showed almost same sensitivity to H2O2 of the wild type root. To know the hormonal effects on the rfrt1-1 root growth, we treated rfrt1-1 root with ABA. rfrt1-1 root showed clear insensitivity to ABA, that treatment apparently inhibited wild type root growth. This insensitivity seemed to derive from the difference of cell length between wild type and rfrt1-1. The RFRT1 expression was also upregulated in the basal meristem and elongation zone by the ABA treatment, but this upregulation was lower than the H2O2 treatment. We also found that H2O2 accumulation in the root by ABA treatment became weaker in the rfrt1-1 than in wild type root.

For identifying RFRT1 target genes, we performed RNA sequencing analysis (RNAseq) by using the wild type and rfrt1-1 roots treated with ABA. As a result of RNAseq analysis, we found 6 genes as the candidates of RFRT1 targets. These expressions were upregulated by both ABA and H2O2. In the rfrt1-1, the induction of the expression of these genes by ABA and H2O2 was diminished strongly. The RFRT1 target gene A (Target A) expressed in the same domain where RFRT1 expressed under the ABA treatment. Moreover, by using Alpha Screen technic, we found that RFRT1 directly bound to promoter region of Target A.

In conclusion, RFRT1 was an important transcriptional regulator in ABA and ROS signaling that regulate plant root growth, and RFRT1 controlled Target A expression that might involve in cell elongation.

Keywords: root growth, transcription factor, ROS, ABA

Poster #189. Shedding light on SCHIZORIZA function in the Arabidopsis root meristem (Submission 415)
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Asymmetric cell division is a key mechanism in generating cellular diversity and patterns in multicellular organisms. The Arabidopsis root meristem is founded during embryogenesis. Its organization is derived from strict asymmetric cell divisions of different stem cells. The root stem cell niche specification and maintenance is controlled by SHORTROOT (SHR) SCARECROW (SCR) and PLETHORA (PLT) transcription factors1,2,3. We identified a novel factor involved in root cell fate separation, the SCHIZORIZA (SCZ) transcription factor4. SCZ acts both cell-autonomously to specify cortical cell identity and non-cell-autonomously to separate cell fates in the surrounding layers. In addition, SCZ is implicated in stem cell formation5 and we have evidence indicating that SCZ acts in parallel with SHR/SCR to specify the root stem cell niche. We investigate this parallel pathway in root niche specification during embryogenesis, using different tissue specific markers. Furthermore, transcriptional profiling was undertaken to identify downstream effectors of SCZ. Results on a number of these SCZ targets will be shown. In addition to these lines of investigation we also performed a suppressor screen, as a powerful tool to identify more functional SCZ targets.

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Keywords: Asymmetric cell division, root stem cell niche, cell differentiation
Poster #190. Heavy Players in Shoot Apical Meristem Maintenance: Elucidating Roles for the WUS-SUMO1 Interaction in Stem Cell Maintenance (Submission 426)
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Plant aerial growth is maintained through the persistence of a set of undifferentiated stem cells located in the shoot apical meristem (SAM) at the apex of the shoot. These stem cells are maintained through the activity of WUSCHEL (WUS), which encodes a homeodomain transcription factor below the stem cell pool in the rib meristem that represses genes responsible for cellular differentiation. WUS also activates CLAVATA3, which encodes a secreted peptide that limits the expression of WUS, creating a negative feedback loop in the SAM to maintain homeostatic levels of WUS and stem cells in the SAM. Recently our lab has shown that WUS is mobile and forms a gradient across the L2 and L1 layers of the SAM, and these functions are required for WUS function and SAM maintenance. This opens new angles for analyzing the mechanisms of WUS mediated SAM maintenance.

To this end, we have performed a yeast two hybrid library screen to identify 118 putative WUS protein interactors using SAM enriched cDNA. One target of interest is the SMALL UBIQUITIN-LIKE MODIFIER 1 (SUMO1), a protein that has been shown to regulate processes such as transcriptional regulation and nuclear/cytoplasmic distribution. Here we provide in vivo evidence that WUS interacts with SUMO1. Further mapping of the interaction shows that SUMO1 interacts across a broad domain of the C-terminus of the WUS protein, which contains domains responsible for both transcriptional regulation and subcellular localization. A broad domain of WUS-SUMO1 interaction suggests that WUS may not be SUMOylated through classical covalent modification but belongs to a class of SUMO interacting proteins. Single knockouts of SUMO1 yield no phenotypes, yet complete knockouts of SUMO1 and its close family member SUMO2 result in embryonic lethality. Lines containing sumo1-1; amiR SUMO2 have been shown to result in developmental patterning defects. Analysis of WUSp::eGFP-WUS in sumo1-1; amiR SUMO2 reveals that the WUS protein was not able to move into the L1 layer of the SAM as it does in WT plants. This suggests the SUMO interaction is required for proper spatial distribution of the WUS protein either by regulating sub-cellular distribution or stability. Alternately SUMO1 interaction may influence transcriptional activity of WUS. To distinguish between these possibilities, we are analyzing the expression of transcriptional targets of WUS, WUS nuclear/cytoplasmic partitioning, stability and mobility in sumo1-1; amiR SUMO2 SAMs.

Keywords: Stem cells, Protein interaction, Development, WUSCHEL, SUMO

Poster #191. AtbZIP63 targets energetic stress response and cell wall growth-related genes (Submission 432)
David Newman¹, Americo Viana¹, Cleverson Matiolli¹, Michel Vincentz¹
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Sugar signalling is vital for a plant's ability to respond to environmental changes in order to optimise growth and maximise its chances of survival. Sucrose-non fermenting Related Kinase(SnRK1) KIN10 mediates responses to energy deprivation and this is thought to be at least partially done through its interaction with the C and S1 class bZIP transcription factors. Analysis of a mutant line of a C class bZIP, atbzip63-2, shows a progressive photoperiod-dependent reduction in growth making it a good candidate for being involved in KIN10-mediated responses. Quantification of the cellular properties of the mutant's Rosette leaves show significant reductions in cell size rather than number. There are also significant changes in the transcript abundance of numerous stress response genes (ASN1, BGAL4, DIN10) and cell wall modification-related genes (five XTH's Xyloglucan Transferase Hydrolyses, 6, 15, 16, 27 and 33, and three expansins, A1, A3 and LA2) when compared to wild-type. Analyses of the expression profiles and growth patterns in AtbZIP63 RNAi knockdown lines and over-expression lines were performed to further examine the function of this gene. Strikingly dysregulation of AtbZIP63 in either direction led to a significant loss of growth in all lines. The expression profiles also showed significant overlap. The target genes common to all the mutant, RNAi and over-expression plant lines were analysed for potential binding sites based on known motifs of the bZIP family. This analysis was used as the basis for performing ChIP assays to determine whether these were direct or indirect targets. We present data that shows in many cases AtbZIP63 directly binds to the promoter regions of these genes. These results make it clear AtbZIP63
has important functions that relate to the optimized growth, development and adaptation to the environment.

Keywords: Arabidopsis thaliana, bZIP transcription factor, sugar signalling, cell wall-modification, abiotic and biotic stress responses

**Poster #192. Balancing activation and repression by a bi-functional transcription factor-WUSCHEL** (Submission 434)

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Stem cell promoting transcription factor (TF)-WUSCHEL synthesized in the rib meristem (RM) migrates into adjacent central zone (CZ) where it promotes stem cell maintenance. WUS functions in direct transcriptional activation of CLV3 and direct repression of differentiation promoting TFs to regulate its own transcript levels and to keep differentiation program repressed in stem cells. How WUS functions both as an activator and a repressor of transcription, and how WUS activates CLV3 specifically in the CZ are not known. Our unpublished data suggests that WUS at higher concentration in the RM repress CLV3 transcription by binding the cis-elements as homodimers. Whereas at lower concentration in the CZ activates CLV3 expression by binding same cis-elements as monomers. Understanding how WUS dimerization on the cis-elements leads to transcriptional repression and monomer binding leads to transcriptional activation requires identification of transcriptional co-regulators that function with WUS. We have identified ULTRAPETALA1 (ULT1) and ULT2, a class of SAND domain containing putative DNA binding proteins, as WUS interacting proteins. We find that both ULT1 and ULT2 interact with homodimerization domains of WUS. This suggested that WUS:ULT heterodimers may promote transcriptional activation. Consistent with hypothesis, CLV3 expression is downregulated in the CZ of ult1 mutants and ectopic activation of WUS in ult1 mutants fails to induce CLV3 expression. In addition to the downregulation of CLV3 in the CZ in ult1 mutants, we also observe an increase in CLV3 expression levels in the RM suggesting that ULT1 function is required for activation of CLV3 in the CZ and repression in the RM. We will present alternate models to explain dual function of ULT proteins in keeping CLV3 expression anchored to the CZ.

Keywords: WUSCHEL, ULTRAPETALA, CLAVATA3, bi-Functional Transcription

**Poster #193. Overexpression of the pollen-essential PIRL9 gene stunts plant growth and suggests a function in sporophyte development** (Submission 439)

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Plant Intracellular Ras-group Leucine-rich repeat proteins (PIRLs) are a plant-specific LRR family structurally related to Ras-interacting LRRs that act in cell signaling and gene regulation in animals. Knockout analysis has demonstrated that Arabidopsis PIRL1 and PIRL9 are functionally redundant and essential in pollen differentiation. PIRLs are also expressed in the sporophyte, but pollen lethality has prevented recovery of double mutant plants. Therefore, to get a more complete picture of PIRL developmental functions, we have over-expressed PIRL9 in transgenic plants. Because LRR domains often mediate highly specific protein interactions in signaling pathways, we predicted that if the target pathway functioned in the sporophyte, PIRL9 overexpression would disrupt development. The PIRL9 cDNA was cloned behind the 35S promoter and introduced into Arabidopsis by Agrobacterium mediated transformation. Multiple transformants were recovered and presence of the insert confirmed by genomic PCR. qRT-PCR revealed substantially elevated PIRL9 mRNA levels. We observed stunted growth, abnormal leaf morphology and occasional anthocyanin accumulation in the T2 and T3 generations in three independent transgenic lines selected for further analysis. PCR of segregating progeny populations confirmed the presence of the transgene in stunted plants. Phenotype severity varied within each line, ranging from seedling lethality to modest growth reduction. Marked segregation distortion was observed in progeny from selfed hemizygotes, indicating likely embryo lethality and/or reduced transmission of the 35S-PIRL9 construct. These results are consistent with a regulatory role for PIRL9 in plant development, and they suggest that PIRL9 binding partners and functional pathways are active in the sporophyte as well as male gametophyte. // Supported by the Whitman College Louis B. Perry Research Fund & an ASPB SURF award.
Keywords: PIRL, leucine-rich repeat, pollen, development, growth, transgenic plants

Poster #194. SCRAMBLED LIKE 1 regulates the epidermal cell patterning in the Arabidopsis root (Submission 450)
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The Arabidopsis root epidermal cell fates are specified in a position-dependent manner. Non-hair cells contact with an underlying single cortical cell while hair cells contact with two cortical cells. In previous studies, it has been shown that several genes, WEREWOLF (WER), CAPRICE (CPC), TRANS PARENT TESTA GLABRA1 (TTG1), GLABRA2 (GL2), and SCRAMBLED (SCM) are involved in the root epidermal cell fate specification. SCM, a receptor-like kinase protein with a predicted extracellular domain regulates the expression of GL2, WER, CPC, and EGL3. By a reverse genetic approach, we found that a gene, SCRAMBLED LIKE 1 (SML1) involves in root epidermal cell fate specification. The sml1 mutant plant shows defects in the root epidermal cell patterning and in the GL2 expression pattern. In addition, other root epidermal cell fate regulators such as CPC, WER and EGL3 also show abnormal expression patterns in the sml1 mutant. These abnormal expression patterns were similar to expression patterns in scm mutant. Therefore, we examined the molecular genetic interaction between SCM and SML1

Keywords: root hair, cell fate specification, SML1

Poster #195. Characterization of expression patterns of XPL genes during Arabidopsis thaliana development (Submission 465)
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In plants phosphatidylcholine (PC) is the most abundant phospholipid in cell membranes. The main biosynthetic pathway for PC depends on phosphocholine (PCho) acting as a precursor in the synthesis. Plants produce PCho via a triple sequential N-methylation of phosphoethanolamine (PEA), reactions performed by the enzyme S-adenosyl-L-methionine:phosphoethanolamine N-methyltransferase (PEAMT). In Arabidopsis thaliana three loci encoding putative PEAMTs have been identified. One of these loci was named XIPO TL1 (XPL1) according to the root phenotype showing swelling at epidermal cells and this encodes an enzyme with demonstrated PEAMT activity. xipotl1 exhibits dramatic changes in root architecture, cell death, and heat-induced male semi sterility that have been associated with decreases in PCho and PC contents. The finding that mutations in XPL1 result in a drastic alteration in the root system development suggests that the function of the members of this gene family is not completely redundant. To demonstrate the effect of the other members of PEAMT gene family, in this work a detailed study of the expression of each member of the XIPOTL gene family is reported. Expression analysis using RT-qPCR showed different patterns for the three XPL genes. XPL1 shows a higher expression in root than shoot tissue, XPL3 expresses in shoot mostly, and XPL2 shows any significant difference between these two tissues analyzed. Transcriptional fusions of the XPL promoter regions with GUS and GFP genes revealed distinct cell-specific expression patterns. Only XPL1 is expressed in floral tissues, mainly in pedicels and the apical region of the carpels. The GFP reporter showed also expression of XPL1 in differentiated columella root cells, cortex cells, and central vasculature at all stages of root development analyzed. While XPL2 showed expression in shoot at early stages, but at six-days old and older seedlings the expression was localized to the hypocotyl, with an unexpected nuclear localization. XPL3 was early expressed after germination in the hypocotyl region but localized to the cell membranes. These findings are consistent with the proposal the Arabidopsis PEAMT genes have non-redundant functions. We are also on the progress of characterizing the phenotype of simple, double and triple mutants to establish the relationship between the three XPL genes and clarify their roles in the root cell development and the PC synthesis.

Keywords: Phospholipids, Phosphoethanolamine N-methyltransferases, XIPOTL, Root cells
Poster #196. Functional analysis in Arabidopsis thaliana of a gene involved in Agave tequilana bulbil development (Submission 472)
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Recently it was reported that the formation of bulbils in Agave tequilana involves de novo formation of vegetative structures through organogenesis in floral pedicel bracts (Abraham-Juarez et al., 2010). The post-embryonic organogenesis is an important ability of plants which allows the physiological adaptation to environmental changes and involves the formation of de novo meristems (Pernisova et al., 2009). Reproduction through bulbils in A. tequilana involves accelerated development of vegetative organs from different structures, so it is likely that there is a complex interaction of genes involved in axillary meristem development. In this project we chose for characterization, one of the genes identified during the early stages of bulbil formation, which was named Mayahuel (May). Due to the tools available in Arabidopsis thaliana, characterization of the two orthologs May1 and May2 was carried out in this species. To date expression analysis in different tissues of Arabidopsis has been carried out, showing that expression is stronger in tissues where cell divisions occur more frequently such as meristems, buds and flowers. Different insertional lines for these genes, have been genotyped, and it was demonstrated that expression of these genes is decreased. Phenotypic analysis of single mutants showed no clear difference to the wild type. Double mutants may1 may2 have been produced, and the phenotypic analysis showed smaller plants, ectopic meristems, multiple rosettes and decreased fertility. Transcriptional fusions with the Uid gene, showed strong expression in shoot apical meristem and root. To explore the possible involvement of the May genes in meristem development expression analysis in May mutants of genes specifying meristem formation was investigated. This analysis showed that STM and BP decrease their expression suggesting that the May genes are associated with the formation and regulation of meristem development and maintenance.

Keywords: Agave tequilana, Bulbils, Shoot apical meristem development

Poster #197. Genetic dissection of the melon gynoecy gene-homologs of in A. thaliana (Submission 485)
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In higher plants, the sexual phenotype of flowers is usually determined by the plant genotype and it can also be modified by various environmental factors and/or internal factors (e.g. hormones). In melon, sex determination is governed by the identity of two alleles at the andromonoecious (a) and gynoecious (g) loci. The gene a encodes for an ethylene biosynthesis enzyme, CmACS-7, that repress stamen development in female flowers. The g gene encodes for a transcription factor, CmWIP1. CmWIP1 gene encodes for a nuclear protein that is conserved throughout the plant kingdom and defines a new subfamily of C2H2 zinc finger (ZF) transcription factor proteins. In Arabidopsis thaliana, six members (AtWIP1 to AtWIP6) have been described as part of this protein family. AtTT1/WIP1 (TRANSPARENT TESTA 1) is involved in the differentiation of seed coat endothelium, and AtNTT/WIP2 (NO TRANSMITTING TRACT) is responsible for the development of the transmitting tract in carpels. The lack of obvious phenotype for the four other insertion mutants affected in the other WIP genes (WIP3, WIP4, WIP5 and WIP6) suggests the existence of functional redundancy within the WIP family in Arabidopsis. Although this class of protein may plays a fundamental role during plant development, the molecular basis of this action remains unclear and no WIP protein has, to date, received a clear functional characterization. To determine redundant activities of the 6 members of WIPs gene family during plant development multiple T-DNA insertion mutants have been produced and a precise phenotyping analysis have been performed using WIP promoter GUS fusions. Simple mutant did not show any new obvious altered phenotype. The phenotyping of the double, triple mutants is in progress.

Keywords: development, no trasmitting tract, wip, transcription factor
Poster #198. Identification of cis-acting elements in the MUTE promoter (Submission 488)
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Stomata are turgor-driven valves on the epidermal surface of land plants that allow for efficient gas and water exchange between a plant and its environment. The formation of stomata begins when an undifferentiated epidermal cell called a meristemoid mother cell (MMC) divides asymmetrically. The smaller daughter cell is called a meristemoid, which renews itself through several rounds of divisions before differentiating into a guard mother cell (GMC). The GMC then divides symmetrically, producing two guard cells surrounding a pore to form a mature stoma.

Many genes have been identified that are required for the progression of stomatal development or patterning, however, very little is known about the transcriptional regulation of these genes. The basic helix-loop-helix (bHLH) protein, MUTE, is both necessary and sufficient for the transition from meristemoid to GMC. We have performed promoter deletion analysis on the MUTE promoter to determine the role of specific cis-elements in the transcriptional regulation of this important master transcription factor. Progress on the characterization of specific cis-elements will be presented.

Keywords: stomata, MUTE, bHLH, cis elements

Poster #199. A link between the SHOOTMERISTEMLESS transcription factor and abiotic stress regulated genes identifies genes involved in growth control. (Submission 500)
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The Arabidopsis SHOOTMERISTEMLESS (STM) homeodomain containing transcription factor is necessary for formation of the shoot apical meristem during embryogenesis (1,2). It is expressed throughout the apical meristem except in cells of incipient leaf primordia where it is turned off. STM mRNA is also expressed in the vascular cambium where it is thought to play a role in maintaining stem cell fate (3). To define the set of genes targeted by STM, and thereby to understand how STM controls meristem formation, we generated a glucocorticoid inducible form of the STM protein. Because we find that attaching epitopes to the carboxy-terminus impairs STM protein activity, we fused the GR epitope to the amino terminus of the STM protein. Indeed, induction of these GR-STM fusion proteins caused the formation of many ectopic meristems, a more dramatic phenotype than that conferred by STM-GR carboxy-terminal fusion proteins (4). Among the genes regulated by STM are many genes previously shown to be induced by a variety of abiotic stresses, including heat, drought, and salt. We have found that a subset of genes regulated both by the developmental regulator STM and by abiotic stresses are involved in growth control.

(3) Groover et al., 2006, Plant Mol Biol 61:917-932.
(4) Gazzani et al., 2004, Science 1046-1048.

Keywords: SHOOTMERISTEMLESS, growth control, shoot apical meristem

Poster #200. Mining genes in the ad/abaxial network for new components of ABA and drought signaling pathways (Submission 501)
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Among the set of genes oppositely regulated by the antagonistically acting REVOLUTA and KANADI genes called ORK genes for OPPOSITELY REGULATED BY REVOLUTA and KANADI - are genes involved in ABA signaling (1). With this in mind, we reasoned that genes of unknown function among the ORK genes might also play a role in ABA signaling. One such ORK gene encodes the ABSCISIC ACID INSENSITIVE VEGETATIVE GROWTH 1 (ABIV1) transcription factor. ABIV1 mRNA increases in response to ABA and also increases in response to drought treatment. Lines homozygous for a loss of function mutation in ABIV1 are resistant to
exogenously added ABA during vegetative stages - plants remain greener, leaves are larger, the shoot apical
meristem is more active and more lateral roots are formed. Furthermore, ectopic expression of ABIV1 causes
changes in vegetative structures that mimic ABA treatment. Thus, ABIV1 appears to act downstream to mediate
changes in development in response to ABA. abiv1 mutant lines, in addition to being resistant to ABA are
unexpectedly resistant to drought as well. We are in the process of characterizing another ORK gene, ORK6,
which encodes a protein of unknown function for its response to ABA. ORK6 protein localizes to the nucleus
suggesting that it also may encode a transcription factor.


Keywords: ABIV1, REVOLUTA, KANADI, leaf polarity, ad/abaxial network, ABA, drought, ORK6

Poster #201. ABA acts upstream of REVOLUTA and KANADI to control germination and meristem activity. (Submission 502)
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HD-ZIPIII transcriptional activators and KANADI transcriptional repressors play opposing roles in polar leaf
development, in promoting shoot apical meristem formation and in regulating outgrowth of organ primordia.
Abscisic acid (ABA) regulates water relations in the plant and promotes seed desiccation tolerance and
dormancy. We now find these regulatory networks show substantial overlap. HD-ZIPIII and KANADI transcription
factors extensively regulate the transcription of ABA signaling genes: multiple members of the PYR-LIKE ABA
receptor, class A PP2C and SNRK3 kinase gene families are controlled by REVOLUTA and/or KANADI1.
Conversely, ABA regulates HD-ZIPIII and KANADI transcript levels and ABA represses a significant subset of
HD-ZIPIII activated and KANADI repressed transcripts. Phenotypic analysis shows that HD-ZIPIII and KANADI
genenes act downstream of ABA on seed germination: HD-ZIPIII proteins promote germination while KANADI
proteins retard germination; HD-ZIPIII renders seed germination more sensitive to red light and less sensitive to
ABA while KANADI does the opposite. Furthermore, KANADI genes and their target ASYMMETRIC LEAVES2 act
downstream of ABA to regulate chlorophyll accumulation and anthocyanin accumulation in response and to inhibit
vegetative. The extensive regulation of ABA signaling genes suggests the link between ABA and HD-ZIPIII and
KANADI is ancient and that the original role of HD-ZIPIII and KANADI transcription factors may have been to
regulate growth in response to environmental stress.

Keywords: HDZIPIII, REVOLUTA, KANADI, ABA, germination, shoot apical meristem

Development: Reproduction including floral development and seed biology

Poster #202. The role of LEUNIG_HOMOLOG in regulating mucilage release from the Arabidopsis testa (Submission 12)
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Upon hydration, Arabidopsis seeds release a pectinaceous mucilage from the seed coat which is primarily
composed of rhamnogalacturonan I (RG-I). Mutation in the transcriptional regulator LEUNIG_HOMOLOG (LUH)
causes a mucilage extrusion defect, which is associated with increased galactose (Gal) residues present on the
RG-I backbone. This structural modification is correlated with reduced expression of MUCILAGE MODIFIED 2
(MUM2), a b-galactosidase belonging to the Glycoside Hydrolase Family 35, raising the possibility that MUM2 is
positively regulated by LUH. As LUH shares significant sequence similarity and functional redundancy with the
transcriptional co-repressor LEUNIG (LUG), it is thought that LUH is likely to function as a transcriptional
repressor and thus regulate MUM2 indirectly by limiting the activity of factors that negatively regulate MUM2. Here
we report on current efforts to define the molecular mechanism of MUM2 regulation and understand how an
increased abundance of Gal residues present on RG-I in luh mutants affects the hydration properties of the
mucilage.

Keywords: LEUNIG_HOMOLOG, mucilage, seed, Gal, RG-I, MUM2, LUH, LUG, transcriptional regulation
To define the regulatory sequences of MUM2, the MUM2 coding sequence was placed under the control of either the MUM2 promoter or MUM2 promoter and large first intron, and introduced into mum2 mutants. This revealed the presence of important regulatory elements in the first intron. Data showing how sequential intron deletions have defined the regulatory elements located in the intron will be presented along with a survey of the potential transcription factor binding sites located within this region. We also report on our experiments to determine whether the increased concentration of Gal residues in luh mutant mucilage prevents pectin-degrading enzymes such as RG lyases (RGLs) from cleaving the RG-I backbone into smaller polymers. Failure to be processed by these enzymes is predicted to alter the hydration properties of the mucilage. To test this hypothesis, we are currently analyzing T-DNA insertions in a family of predicted RGLs that are expressed in the seed coat and will present the results of this analysis. In addition, we report on the consequences of over-expressing selected RGLs in either mum2 or luh-1 mutant backgrounds. Results from this study lay the foundations for understanding the transcriptional control of pectin modification and further define the role of the LUG-class of transcriptional co-repressors in plants.

Keywords: LEUNIG_HOMOLOG, Arabidopsis Seed Coat, Mucilage extrusion

**Talk #203. Maternal temperature history activates Flowering Locus T in fruits to control progeny dormancy according to time of year** (Submission 31)

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Seasonal behavior is important for fitness in temperate environments but it is unclear how progeny gain their initial seasonal entrainment. Plants use temperature signals to measure time of year, and changes to life histories are therefore an important consequence of climate change. Here we show that in Arabidopsis the current and prior temperature experience of the mother plant is used to control germination of progeny seeds, via the activation of the florigen Flowering locus T (FT) in fruit tissues. We demonstrate that maternal past and current temperature experience are transduced to FT, which controls seed dormancy through inhibition of proanthocyanidin synthesis in fruits. Transport of proanthocyanidin to seeds promotes dormancy by increasing seed coat tannin content. Our data reveal that maternal temperature history is integrated through FT in the fruit to modulate a metabolic signal that entrains the behavior of progeny seeds according to time of year.

Keywords: seed dormancy, flowering time, seed germination, temperature, transgenerational signalling, phenylpropanoid metabolism

**Poster #204. Glyoxalase I at the nexus of compatible and self-incompatible pollinations in Brassica** (Submission 66)

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In Brassica sp., self-incompatibility (rejection of self-pollen) is a genetic mechanism resulting from a haplotype specific interaction between the pollen-specific small cysteine-rich secreted protein (SP11/SCR) and stigma-specific S-receptor kinase (SRK). After the attachment of 'self' pollen to the papillary cells of the stigma, SCR/SP11 binds to the membrane localized SRK, and this receptor-ligand interaction leads to activation of SRK. A phosphorylation cascade follows that activates the ARC1 E3 ubiquitin ligase, leading to the ubiquitination (Ub) and proteasomal degradation of compatibility factors needed for the pollen tube to grow. While our understanding of the interactions between SRK and SP11/SCR is quite extensive, information pertaining to the subsequent signaling pathway that leads to the rejection response has remained unexplored. We performed a 2D DIGE analysis to decipher the proteins down-regulated during self-incompatible pollination in brassica and probable targets of ARC1. One of the candidate proteins identified by this approach was S-lactoylglutathione lyase (Glyoxalase I or GLO1). GLO1 is involved in the detoxification of methylglyoxal (pyruvate aldehyde), an endogenous cytotoxic compound formed as a byproduct of glycolysis. Methylglyoxal has high reactivity in glycation reactions in vivo, resulting in the formation of AGEs (advanced glycation end-products) of protein, nucleotides and basic phospholipids. Thus, Glyoxalase-mediated detoxification of methylglyoxal is essential for
cellular homeostasis. Given the protective function of GLO1, we hypothesized that following an SI interaction, targeting of GLO1 for degradation would result in increased methylglyoxal levels in the papillary cells, causing sudden increase in cytotoxicity to the system leading to a pollen rejection response. BnGLO1::RFP accumulated in Arabidopsis stigmas following compatible pollinations. RNAi-mediated suppression of GLO1 in compatible Westar lines resulted in significant reduction in pollen attachment and seed set, while over-expression of GLO1 in self-incompatible W1 lines led to partial break down of incompatibility. Our observations indicate that GLO1 is an essential compatibility factor which is likely targeted for degradation following an incompatible response.

Keywords: Self-incompatibility, Brassica, Glyoxalase I

Talk #205. Regulation of Arabidopsis flower development by AIL/PLT transcription factors (Submission 87)
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Flowers initiate in the periphery of the shoot apical meristem, a dome-shaped structure at the apex of a plant that allows for the initiation of new organs throughout the plant's life. After outgrowth of a flower primordium from the meristem, floral organ primordia appear in characteristic positions within the flower and undergo coordinated cellular behaviors and morphogenesis to develop into one of four organ types. Little is known about the mechanisms that dictate the positioning of floral organ primordia at precise locations within the flower and the means by which cell division, growth and differentiation are coordinated during floral organogenesis. Members of the Arabidopsis AIL/PLT gene family play partially overlapping roles in several aspects of flower development including floral organ initiation, growth and patterning. While only loss of ANT function results in a floral phenotype, loss of AIL5, AIL6 or AIL7 in combination with loss of ANT activity produces double mutants that exhibit more severe defects than ant single mutants. Distinct phenotypes are also observed in different ail triple mutant combinations. These genetic studies also reveal dose-dependent activities of AIL6 and AIL7 in certain mutant backgrounds. Transcriptomic studies on ant ail6 double mutants suggest important roles for these proteins in cell wall remodeling and growth. In addition, there appears to be cross-regulation of AIL gene expression.

Keywords: flower development, AIL/PLT proteins, floral organogenesis

Poster #206. Expression of Arabidopsis thaliana HB17 gene in corn leads to improved sink potential (Submission 89)
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As a result of the large scale screening of candidate genes in transgenic corn, we identified an Arabidopsis thaliana gene HB17, a member of homeodomain-leucine zipper II (HD-Zip II) family of the plant transcriptional factors, which affects plant growth and leads to increase in ear size at silking. When expressed in corn, AtHB17 lacks the repression domain due to the corn-specific splicing mechanism and loses the ability to bind the co-repressors and affect transcription of the target genes. The protein still can form homo-dimers as well as hetero-dimers with corn endogenous HD-Zip II proteins and bind to the target DNA sequences due to the presence of the functional leucine-zipper and DNA-binding domains. We propose that AtHB17 expressed in corn mediates physiological effects through dominant-negative mechanism by attenuating transcriptional repression activity of endogenous corn HD-Zip II proteins. We hypothesize that modulation of the activity of HD-ZIP II proteins leads to modulation of corn plant's growth responses to environmental and developmental signals and, ultimately, to increased ear size, thus, providing opportunity for enhanced sink potential in corn plants. Increased sink potential could be manifested through an increase in kernel weight or kernel number depending on the environmental conditions.

Keywords: transcription factor, HD-Zip, yield, corn, sink potential, kernel
Poster #207. SCARECROW-LIKE 15 interacts with HISTONE DEACETYLASE19 and is essential to the repression of the seed maturation program (Submission 90)

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In eukaryotes, histone deacetylase (HDAC) forms a multimeric protein complex in which the associated factors facilitate HDAC enzymatic activity. The seed maturation program is repressed after seed germination and during seedling establishment. HDAC6 and HDA19 redundantly contribute to the repression of embryonic properties after germination, but the mechanism is obscure. Here, we report a HISTONE DEACETYLASE19 (HDA19)-associated transcriptional coregulator, SCARECROW-Like15 (SCL15), that is critical in the repression of seed maturation genes in Arabidopsis seedlings. SCL15 was localized in nucleus and physically interacted with HDA19. SCL15 promoter activity and GFP-tagged SCL15 predominantly localized in the abaxial (phloem) side of the vascular bundles, particularly in the phloem companion cells and neighbouring pericycle cells in roots, placentochalaza cells in developing seeds and bundle-sheath cells in leaves. This expression pattern of SCL15 is largely consistent with the previously reported accumulation and subcellular localization of storage reserves in vegetative tissues. Mutation of SCL15 affected plant growth and led to a global shift in gene expression to a profile resembling late embryogenesis. A large subset of genes involved in the seed maturation program were markedly derepressed in scl15 mutant seedlings, including those encoding seed storage proteins and seed-specific vacuolar processing enzymes. Consistent with this, scl15 mutant seedlings accumulated 12S globulin in an ABA-dependent manner and an increased level of H3 acetylation at a subset of embryonic genes was found in scl15 plants. These phenotypes of SCL15 mutation were fully restored by complementation with SCL15pro::SCL15:GFP. These data suggest that during vegetative growth in Arabidopsis, SCL15 acts as a repressor to form a complex with HDA19 for preventing the expression of embryonic traits in vegetative tissues.

Keywords: Seed maturation, Seed gene repression, SCARECROW-like15, Histone deacetylase19, Transition of development, Embryonic traits in seedlings

Poster #208. Paradigm shift: Extreme functional redundancy at the MKK level in control of pollination by mitogen activated protein kinase cascade in Arabidopsis (Submission 102)

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Mitogen activated protein kinase (MAPK) based signaling network controls diverse aspects of plant growth and development. MAPK family members are known to function in development of reproductive organs such as pollen, anther, stigma and ovule development in Arabidopsis. In Arabidopsis, pollination is a highly coordinated process, which relies on proper communication and signaling between pollen and the stigmatic papillae. We have identified a functionally redundant MAPK matrix in controlling pollination responses in Arabidopsis. Through a combination of null mutants and stigma-specific RNAi mutants, we have identified the MAPKs and the upstream interacting MAPKKs required for successful pollen attachment and pollen tube growth on naturally pollinated stigmatic papillae. In contrast to the currently accepted paradigm where the 10 MKKs form the bottle-neck through which multiple MKKs signal to more than 20 MPKs, we have observed a role reversal where multiple MKKs signal to a limited set of MPKs. Taken together, our results demonstrate the involvement of multiple MKKs and MAPKs in regulating pollination responses in Arabidopsis.

Keywords: MAPK: Mitogen-activated protein kinase, Pollination, Arabidopsis

Poster #209. A Screen for MUCILAGE-RELATED Genes Reveals Novel Players in Cell Wall Biosynthesis (Submission 112)

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The epidermal cells of the Arabidopsis thaliana seed coat synthesize copious amounts of cell wall polysaccharides at a very specific stage of development [1]. Mature seeds release these polymers as a large mucilage capsule upon hydration. Although mucilage could facilitate seed dispersal and germination, its exact biological function has not been clearly established.

In the past decade, the Arabidopsis seed epidermis has become an increasingly popular model for the discovery of cell wall genes, since the mucilage it produces can be directly examined using cell wall stains or easily extracted for chemical analysis. To date, only a few genes involved in seed coat mucilage biosynthesis have been identified and characterized in detail.

At least nine genes (RHM2/MUM4, MUM2/BGAL6, SBT1.7/ARA12, PMEI6, FLY1, BXL1, GL2, GATL5, PER36) that were previously shown to control the structure and properties of mucilage are preferentially expressed in the seed coat during its biosynthesis [2,3]. We used the expression profile of these genes and public microarray data to identify MUCILAGE-RELATED (MUCI) genes. We found more than 100 MUCI genes, many of which are members of the Carbohydrate-Active enZYmes Database (www.cazy.org). We screened more than 250 muci T-DNA insertion mutants using mucilage stains and mucilage compositional analysis.

The MUCI screen led to the identification of multiple MUCI genes that are required for proper mucilage structure and release, including several previously uncharacterized glycosyltransferases. Interestingly, the characterization of the muci mutants showed that even changes in minor mucilage components can dramatically alter mucilage properties. The study of these mutants gives us the unique opportunity to explore the functional roles of individual polymers in mucilage organization.

Keywords: cell wall biology, seed coat development, mucilage biosynthesis, co-expression analysis

Poster #210. UNDERSTANDING THE REGULATION OF FLOWERING-TIME IN LEGUMES (Submission 133)
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Legumes are the second most important crop family worldwide and contribute more than 33% of human dietary protein. As flowering time is a key trait in most legume breeding programmes, we are using Medicago truncatula as a model legume to study how flowering time is regulated.

Arabidopsis thaliana has provided fundamental information about genes and genetic pathways controlling the flowering process. FT (the florigen) is expressed in leaves and translocates to the shoot apical meristem where it binds to FD, a bZIP transcriptional factor. The complex of FT-FD activates genes, such as SOC1, which control the floral transition. Five FT homologs have been identified in M. truncatula and at least two of them (MtFTa1 and MtFTb1) function as the key floral regulators. Moreover, an FD and three SOC1 homologs have been identified in M. truncatula.

To investigate how FT controls Medicago flowering, we screened a retrotransposon (Tnt1) mutagenized population of M.truncatula and identified fd mutants. The fd mutants flowered significantly later than wild-type, indicating that FD functions in flowering. To investigate the downstream regulators of MtFT/MtFD, fd and fta1 mutants were analysed using RT-PCR. This data indicated that, as in Arabidopsis, SOC1 genes are involved in flowering time control of Medicago and function downstream of FT/FD.

Keywords: flowering time, legumes, medicago, FD, SOC1

Poster #211. Regulation of Proteasome Activity by Abscisic Acid and High Temperature during germination in Arabidopsis (Submission 137)
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Primary dormancy is introduced during the final stages of embryogenesis and is accompanied by a rise in abscisic acid (ABA). Seeds integrate endogenous and environment signals, such as light, temperature, and nutrient availability, to break dormancy and trigger germination. Abiotic stresses, such as high temperature, increase de novo ABA synthesis and introduce secondary dormancy to inhibit germination. Changes in ABA levels alter the stability and levels of many proteins involved in ABA signalling and dormancy regulation through the proteasome pathway (1). However, the role and activity of the proteasome during germination and in response to hormonal and environments signals is poorly understood. FUSCA3, a transcription factor required for dormancy establishment during late embryogenesis, is induced during imbibition at high temperature to inhibit germination in Arabidopsis (2). FUSCA3 is an unstable protein, rapidly degraded by the proteasome and stabilized in the presence of ABA (3). Here, we characterize the activity of the proteasome during germination at high temperature and in the presence of ABA, by monitoring the degradation of FUSCA3 and the cleavage of a synthetic proteasome substrate in vitro. Our results indicate that proteasome activity gradually increases during germination at optimal temperature. High temperature and exogenous ABA have an inhibitory effect on proteasome activity during germination, while inhibition of ABA synthesis pharmacologically or genetically increases proteasome activity. Furthermore, inhibition of ABA-mediated proteasome activity is developmentally regulated and restricted to the first few days of germination. In conclusion, our data suggests that a combination of environmental and hormonal signals contribute to regulate the activity of the proteasome during germination.

REFERENCES

Keywords: seed germination, ABA, high temperature, proteasome

Poster #212. Mutations in the RUBY gene can suppress the defective mucilage extrusion phenotype of mum2 mutants. (Submission 138)
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In myxospermous species such as Arabidopsis, when seeds are exposed to an aqueous solution, pectin-rich mucilage is extruded from the seed coat to envelop the seed. The seed coat mucilage of Arabidopsis provides an excellent model for studying the synthesis, structure and function of pectin, since mucilage is not essential to plant growth or fertility, and it is easy to extract and study. When wild-type Arabidopsis seeds are hydrated in Ruthenium Red the extruded mucilage can be seen as a pink-coloured halo surrounding the seed. Mutants that fail to extrude mucilage were isolated, and we have used genetic suppressor analysis of one of these mutants, mum2, to identify other genes that are involved in mucilage modification. One of the mum2 suppressors we have identified has been named ruby because of its distinctive phenotype. Mutations in the RUBY gene allow mucilage to extrude from mum2 seeds but the extruded mucilage contains particles that stain more darkly with Ruthenium Red than the majority of the mucilage. Microscopic analysis of these particles indicates that they may be whole cells of the outer epidermal layer that have separated from the inner layers of the seed coat, but remain attached to the seed because they are embedded in the mucilage. This phenotype suggests that RUBY may be needed to strengthen the connections between the cells of the outer and inner epidermal layers of the seed coat. The ruby phenotype is observed whether or not the mum2 mutation is present in the background. The suppression of the mum2 phenotype by ruby mutations indicates that RUBY likely has functions affecting the structure of pectin in the mucilage or outer cell wall as well. Cloning of RUBY has shown that it encodes a glyoxal oxidase-related protein (GLOX). GLOX genes have not been well-studied in plants, but they have been shown to be up-regulated in response to defense signals. We are studying the expression of the RUBY gene in Arabidopsis at the genetic and biochemical level in order to elucidate its function in pectin and cell wall modification. In addition, we are examining the genetic interactions of this gene and other genes known to be involved in pectin structure and
modification with the hope of creating a more complete picture of how this complex family of polysaccharides affects cell walls and other pectin-containing structures in plants.

Keywords: seed coat, cell wall, mucilage, suppressor, genetics, pectin, mutant

Poster/Talk #213. Different mechanisms underlie the regulation of floral transition by a pair of highly related but non-redundant Ubiquitin E3 ligase genes (Submission 159)
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In plants, flowering marks the transition from vegetative to reproductive development, and is coordinated with favorable environmental conditions. Plants have thus evolved intricate mechanisms to control floral transition. The photoperiodic flowering pathway perceives signals of day length and light quality to regulate the expression of several flowering-time genes and the stability of their translational products. We have recently implicated small protein modifiers in the regulation of photoperiodic flowering by identifying CDF2, a known floral repressor, as a substrate for a novel SUMO-Targeted Ub ligase (AT-STUbL4 or STL4). Here, we show that STL4 belongs to a group of genes that arose by duplication events dating prior to the divergence of Arabidopsis from other Brassica species ~ 43 MYA. Despite a high degree of sequence relatedness, STL4’s closest paralogue (STL4-Like or STL4L) is unlikely to act as a STUbL since it does not complement yeast stubl mutants. Mutations and RNAi knockdown lines of either gene flower later that wild-type plants, whereas their over-expressor lines flower earlier, suggesting that both genes promote flowering. However, the two genes regulate the expression of different flowering-time genes, and hence they may act on different protein targets. Consistent with this, double mutants that combine a mutation of one gene and an RNAi transgene that down-regulates the expression of the other exhibit additive effects, flowering much later than either single mutants. Additionally, reciprocal rescue experiments suggest that expression of either gene in the mutant background of the other does not rescue the late-flowering phenotype of that mutant. These experiments suggest that despite common evolutionary origin, sequence relatedness and a function in floral transition, these two genes likely diverged enough to act on different flowering-time proteins and hence to regulate floral transition by different mechanisms. Current experiments attempt to genetically place STL4L in the appropriate flowering pathways.

Keywords: SUMO, Ubiquitin, Flowering Time, STUbL

Poster #214. Characterization of ISE2-mediated RNA Editing in Arabidopsis (Submission 174)
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INCREASED SIZE EXCLUSION LIMIT 2 (ISE2) is a nuclear gene encoding a chloroplast-localized DEVH RNA helicase required for Arabidopsis embryogenesis. ISE2 plays essential roles in the processing of chloroplast rRNA species, splicing of group II introns and in transcription, which is consistent with general functions of RNA helicases. Screens to identify protein partners of ISE2 isolated a putative Pentatricopeptide Repeat Protein (PPR) protein encoded by At5g03800, which we called IPI1 (for ISE2 protein interactor 1). PPR proteins are known function in RNA editing. However, ISE2’s role in chloroplast editing is unknown. Using primer pairs specific to known editing sites within Arabidopsis chloroplast transcripts, RNA was isolated from wild type Arabidopsis plants and from plants with reduced ISE2 expression due to cosuppression, and editing monitored. The data indicate that ISE2 is required for RNA editing of several chloroplast transcripts, including transcripts which function in photosynthetic electron transport, protein degradation and chloroplast transcription. Current experiments are aimed towards: (i) examining whether IPI1 plays a role in editing the same transcripts as ISE2 (ii) further characterizing the putative interaction between ISE2 and IPI1 and (iii) characterizing the functional consequences of the editing defects in ise2 mutants.

Keywords: RNA Editing, ISE2, PPR, Embryogenesis, Chloroplast, RNA Metabolism
Poster/Talk #215. Non-equivalent contributions of maternal and paternal genomes to early plant embryogenesis (Submission 180)
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Zygotic genome activation in metazoans typically occurs several hours to a day after fertilization, and thus maternal RNAs and proteins drive early animal embryo development. In plants, despite a number of molecular studies of post-fertilization transcriptional activation, the timing of zygotic genome activation remains unclear. For example, two recent reports that used different hybrid ecotype combinations for RNAseq profiling of early Arabidopsis embryo transcriptomes came to divergent conclusions. One identified paternal contributions that varied by gene, but with overall maternal dominance, while the other found that the maternal and paternal genomes are transcriptionally equivalent. To functionally assess paternal gene activation in an isogenic background, we performed a large-scale genetic analysis of 49 EMBRYO DEFECTIVE (EMB) genes, testing the ability of wild type paternal alleles to complement phenotypes conditioned by mutant maternal alleles. Our results demonstrate that wt paternal alleles for 9 of these genes are completely functional 2 days after pollination (dap), with the remaining 40 genes showing partial activity beginning at 2, 3 or 5 dap. Using our functional assay, we also demonstrate that different hybrid combinations exhibit significant variation in paternal allele activation, reconciling the apparently contradictory results of previous transcriptional studies. The variation in timing of gene function that we observe shows that paternal genome activation does not occur in one early discrete step, demonstrates that maternal and paternal genomes make functionally non-equivalent contributions to early plant embryogenesis, and uncovers an unexpectedly large effect of hybrid genetic backgrounds on paternal gene activity.

Keywords: embryo, gene activation, hybrid

Poster #216. A putative maternal polarity complex for posterior (chalazal) endosperm development in Arabidopsis (Submission 185)
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Formins are actin-nucleators with conserved functions in developmental processes such as cell polarity, cytokinesis, and morphogenesis. The Arabidopsis formin AtFH5 is maternally expressed in the posterior pole of the seed endosperm and is involved in the production of the chalazal cyst. This maternal-posterior pattern of expression requires the FIS Polycomb histone-methyltransferase. The mechanisms for plant formin localization and activation, however, are potentially unique within eukaryotes. In yeast and metazoans, N-terminal formin domains provide membrane localization and activation through interaction with Rho-GTPase. Plants, however, possess a distinct sub-family of Rho-like GTPases (ROPs) and the plant N-terminus is highly diverged including a membrane localization signal. A yeast two-hybrid screen against AtFH5 identified FLA12, ROP2 and AtFH5 itself. We characterized a new allele of ROP2, rop2-2, that appears to be a strong allele in comparison to rop2-1. Rop2-2 displayed mutant phenotypes in the growing pollen tube tip, a site of AtFH5 expression, and in the seed. Similar to AtFH5, we primarily detect only maternal expression of ROP2 in the developing seed using paternally expressed PHERES1 transcripts as a control for paternal mRNA abundance. However, certain ecotypes either retain biallelic expression of ROP2 or possibly lack a maternal competence to inhibit paternal gene expression. We are currently examining rop2, atfh5 double mutants. Preliminary results that ROP2 acts upstream of AtFH5.

Keywords: seed endosperm, formin, cell polarity, genomic imprinting

Poster #217. Functional differences in two Arabidopsis AIL/PLT transcription factors result primarily from differences in their expression levels and domains (Submission 186)
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AINTEGUMENTA (ANT), a transcription factor of the AINTEGUMENTA-LIKE/PLETHORA (AIL/PLT) family, regulates floral organ size in Arabidopsis thaliana. ant mutants produce smaller floral organs while ANT overexpression lines produce larger flowers. A related gene, AINTEGUMENTA-LIKE6 (AIL6), shares high
sequence similarity with ANT in its DNA-binding domain, which is composed of two AP2 DNA repeats and an intervening linker region. ail6 single mutants have wild-type flowers but ant ail6 double mutants produce flowers that lack petals, stamens and a normal gynoecium. The more severe floral defects in ant ail6 double mutants as compared to ant single mutants indicate that ANT and AIL6 have partially redundant function in flower development. We have investigated the molecular basis for the functional differences in ANT and AIL6 that may be responsible for the inability of AIL6 to replace ANT function with regard to floral organ growth. These functional differences could result from differences in the expression patterns of these two proteins and/or differences in their protein activities. In flowers, ANT mRNA is detected in a broader domain and at higher levels compared to AIL6 mRNA. We made transgenic plants in which AIL6 is expressed under the control of the ANT promoter in an ant mutant background (i.e. ANT:gAIL6 ant). Rescue of the ant mutant phenotype in these lines would indicate that the functional differences between ANT and AIL6 are due to differences in the expression of these genes. We find that the ANT:gAIL6 transgene can rescue the floral organ size defect of ant mutants, however in some lines we observed additional phenotype that include reduced numbers of floral organs and the production of mosaic organs. We hypothesize that this may result from higher levels of AIL6 expression in these lines and that high AIL6 activity may regulate genes that are not targets of ANT regulation.

Keywords: flower development, AIL/PTL transcription factor, redundancy, floral organ size, loss of floral organs

Poster #218. Create the scene and watch the show unfold: Identification of seed genes repressors in Arabidopsis Seedlings (Submission 196)
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Seed maturation is a highly regulated developmental phase when the embryos accumulate high quantities of storage compounds, including seed storage proteins (SSPs), to provide a source of nutrition for the seedlings to develop after germination. Genes encoding SSPs are specifically and highly expressed in the seed during maturation. However, the mechanisms that repress the expression of these genes in vegetative tissues are not well understood. To identify the repressors of SSP in vegetative organs, transgenic Arabidopsis plants expressing the GUS reporter gene under the control of a SSP gene promoter from soybean (Glycine max), were generated. Experiments with the transgenic line confirmed that GUS is specifically expressed in embryos and not detectable in vegetative tissues. Homozygous transgenic plants were mutagenized with ethyl methanesulfonate (EMS) and the progeny of mutated plants were screened for individuals displaying ectopic expression of seed storage protein genes (essp) in leaves. Two mutant lines, essp7 and essp8, were used in this study. essp7 plants show pleiotropic developmental defects including multiple shoots and branches, short stature, late flowering, short siliques, and somatic embryo formation at a very low penetrance (~1%). essp8 mutation was shown to be lethal. The seedlings are extremely small which can form somatic embryo at low penetrance (~10%). Genetic analysis revealed that essp7 and essp8 mutations are both recessive. Bulked-segregant analysis with simple sequence length polymorphism (SSLP) markers, located the mutations on chromosome 1 and chromosome 3 in essp7, and on chromosome 2 in essp8. Next-generation mapping (NGM) was used to detect the genes responsible for the observed mutant phenotypes in the mapped regions. essp7 and essp8 plants were sequenced using 100nt paired-end sequencing on the Illumina HiSeq and 300nt paired-end sequencing on the Illumina MiSeq, respectively. The NGM results were consistent with prior mapping results for both mutants. All non-synonymous EMS mutations identified within the candidate regions were detected and confirmed using co-segregation test, allelism test, generation of knockout lines for the genes of interest, and genetic complementation of mutant phenotype. NGM could provide high-resolution genotypic information that can be used as a complement for traditional map-based cloning by direct identification of mutations in a fast and cost-effective way.

Keywords: Seed Storage Proteins (SSPs), Ectopic Expression of Seed Storage Proteins (essp), Next-generation Mapping (NGM)
Poster/Talk #219. Egg cell number and cell type differentiation are dependent on cytokinin in the Arabidopsis female gametophyte. (Submission 230)
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In flowering plants, reproduction occurs through double fertilization involving the gametes produced by the haploid gametophytes. The Arabidopsis female gametophyte (or embryo sac) embedded in the ovule has a well organized structure with 2 gametes, which are the egg and the central cell, and 5 accessory cells. Producing the correct number of nuclei and specification of cell identities are critical to form the functional embryo sac. While auxin has been shown to be involved in female gametophyte development, the role of other phytohormones is still unknown. Here, we used a cytokinin biosynthesis gene AtIPT8 and a cytokinin oxidase gene AtCKX1 to increase and degrade the cytokinin within the embryo sac, respectively.
In cytokinin depleted embryo sacs, excessive nuclei are produced at the egg cell position. We show that these excessive nuclei develop into functional egg cells that can be fertilized and progress into twin embryos expressing a paternally derived marker gene. The extra functional egg cells are a product of excessive nuclear division rather than a specification switch from other cell types in the embryo sac. Random supernumerary nuclei are also observed in some ovules in the same line, which resemble the reported RBR (Retinoblastoma-related) null mutant phenotypes. Considering the coordinated regulation of cytokinin and RBR for cell division during root development, our results imply cytokinin is likely regulating the female gametophyte cell division through a RBR-related pathway.
In embryo sacs with elevated cytokinin levels, we observed delayed cellularization, aberrantly enlarged nuclear sizes, and mis-expression of cell-specific markers, indicating defective cellular differentiation. Because plant cell differentiation relies on antagonistic activities between cytokinin and auxin, we hypothesize that the role of cytokinin during the embryo sac differentiation is accomplished by interacting with auxin.
Taken together, our results suggest that cytokinin is essential for female gametophyte development by determining nuclear number and cellular differentiation. Lower levels of cytokinin will trigger abnormal nuclear division at the egg cell position, whereas excessive cytokinin tends to affect cell differentiation during female gametophyte development. The underlying mechanisms likely involve two distinct interacting pathways: cytokinin-RBR for nuclear division and cytokinin-auxin for cell differentiation.

Keywords: reproductive development, Female gametophyte, Egg cell, cell specification, Cytokinin

Poster #220. Regulation of maize seed filling: the role of ZmZOU in embryo-endosperm communication (Submission 243)
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In angiosperms, the seed is composed of three major compartments: the maternally derived seed coat, an embryo and an endosperm. Although the development of each seed compartment is controlled by distinct genetic programs, normal seed development also demands coordination, and thus communication between compartments. In Arabidopsis, the bHLH transcription factor ZHOUPI (ZOU) is involved in embryo-endosperm communication (Yang et al., 2008). ZOU regulates endosperm breakdown and mature atzou seeds therefore have a persistent endosperm and consequently a reduced embryo development. ZOU also controls the formation of the cuticle on the embryonic surface via the ALE1/GSO1GSO2 signaling pathway (Xing et al., 2013). AtZOU, together with its partner INDUCER OF CBP EXPRESSION 1 (AtICE1) directly regulates directly the expression of target genes in the developing endosperm (Denay et al, 2014).
In contrast to the Arabidopsis endosperm, which is normally a transitory structure, maize seeds contain an endosperm which is prominent and persistent. The maize genome contains a single gene in the same phylogenetic clade as AtZOU, which we have named ZmZOU. The deduced amino acid sequence of ZmZOU has an N-terminal extension of 300 amino acids, which is conserved to an increasing extent in rice and sorghum. The overall sequence similarity of 54% between AtZOU and ZmZOU increases to 86% in the conserved bHLH domain. ZmZOU expression is strictly limited to the endosperm where it peaks during the filling stage. ZmZOU-RNAi lines show subtle defects in embryo development and in a specific domain of the endosperm. The analysis
of RNAseq data from wild-type and transgenic seeds has allowed the identification of potential ZmZOU target genes involved in endosperm development. We show that ICE-like proteins from maize can bind to ZmZOU and drive expression from specific target promoters. Ectopic expression of ZmZOU causes defects during vegetative development, likely due to inappropriate binding with its partners. Finally, complementation of the atzou mutant by ZmZOU allows a marked but incomplete recover of wild-type phenotypes and target gene expression. The comparative analysis of the ZOU/ICE complex of transcription factors in maize and Arabidopsis may further our understanding of the differences in endosperm breakdown observed in these two species.

Keywords: Endosperm, Seed, Maize

Poster #221. Connecting the role of Arabidopsis BPM proteins in CUL3-based E3 ligases to Fatty Acid Metabolism in Plants (Submission 249)

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E3 ubiquitin protein ligases play important regulatory roles in cell division, cell cycle control and general development in eukaryotic organisms. CULLIN3 (CUL3) and MATH-BTB/POZ domain (BPM) proteins form CUL3-based ubiquitin E3 ligases (CRL3BPM) complexes that facilitate ubiquitination of target proteins, which can lead to their degradation via the 26S proteasome. Arabidopsis thaliana encodes for six BPM (BPM1 to 6) and two CUL3 proteins (CUL3a and CUL3b). It has been shown that BPM proteins are able to interact with a variety of members of the ERF/AP2 (ethylene-responsive factors/ APETLALA2) transcription factor family. ERF/AP2 proteins comprise more than 140 members in Arabidopsis, and are involved in a wide variety of developmental and physiological processes. By employing a combined set of biochemical, molecular, and genetic studies we can show that CRL3BPM E3 ligases target the ERF/AP2 protein WRINKLED1 (WRI1), a key regulator of fatty acid metabolism in seeds, for ubiquitylation and subsequent degradation via the 26S proteasome. Phenotypic studies of BPM loss-of-function plants (6xamiBPM) show wide defects in development and fatty acid metabolism. Overall these results define CRL3BPM E3 ligases as central and novel regulators in controlling transcriptional and metabolic processes in Arabidopsis through ERF/AP2 proteins, and emphasize their importance for whole plant development.

Keywords: CUL3, WRI1, Ubiquitin proteasome pathway, Fatty acid biosynthesis

Poster #222. Natural variation in epigenetic pathways affects the specification of female gamete precursors in Arabidopsis (Submission 279)

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Although in most flowering plants a single cell is specified as the gametic precursor, many other species exhibit variation in the origin and number of precursor cells that participate in female gametogenesis. We systematically analyzed megasporogenesis in Arabidopsis thaliana and found that the mechanisms that specify differentiated pre-meiotic cells are naturally variable. We also found differences in the pattern of expression of epigenetic proteins involved in the control of cell specification. Intra-specific hybridization and polyploidy produced perturbations similar to those found in mutants of previously characterized small RNA pathways, suggesting that their influence on megasporogenesis might depend on the action of these epigenetic mechanisms. Our results suggest that differences in the epigenetic control of cell specification lead to natural phenotypic variation during megasporogenesis in Arabidopsis, a mechanism that could serve as the starting point for the emergence and evolution of natural reproductive alternatives occurring in the ovule of flowering plants.

Keywords: Megasporogenesis, Natural Variation, Gametic cell specification
In spite of its agricultural importance, the mechanism of anther development is not well understood. The majority of cell types in plants have cell walls, some of the exceptions being tapetal cells, egg cells and sperm cells. Here, we report that the tapetal cells of the anther of Arabidopsis thaliana did not appear to have a cellulosic wall based on staining with Calcofluor and Renaissance 2200 at microspore stage. At anther stage 4, when the four-lobed anther pattern is generated and tapetal cells appear, all the anther cell layers including tapetal and sporogenous cells had Renaissance 2200 fluorescence in the cell walls. However, at stage 5, when all types of cells that compose the anther are present, the epidermis, endothecium and middle layer had strong fluorescence, while the tapetal cells and the microsporocytes had faint fluorescence. Thus, these data show that cellulosic walls on the tapetal cells and microsporocytes were degraded at stage 5 in the wild-type anthers. To determine whether genes regulating anther development are involved in cellulosic wall degradation, we examined changes in cellulosic component in a sporocyteless/nozzle (spl/nzz) mutant, in which anther development stops before stage 5 and neither microsporocytes nor anther walls are formed. In spl/nzz mutant, at stage 3, all the anther cell layers including primary parietal cell and primary sporogenous cell layers specifically fluoresced in the cell walls. In the matured anther all cells also specifically fluoresced in the cell walls. These data show that cellulosic wall degradation occurs downstream of anther tissue differentiation regulated by SPL/NZZ. Abnormal anther development occurs later in the tapetum determinant1 (tpd1) mutant than in the spl/nzz mutant. In the tpd1 mutant, anthers lack the tapetum but produce more microsporocytes. At stage 3, the cell walls of the epidermal cells, cells of the secondary parietal cell layers and sporogenous cells showed Renaissance fluorescence. At stage 5, the cell walls of the epidermis, endothecium and middle layer were strongly fluorescent but the cell walls of the microsporocytes showed almost no fluorescence similar to the wild type. These results suggest that the tapetum cells and microsporocytes lose cellulosic walls during microsporocyte formation, and that cell wall degradation occurs downstream of SPL/NZZ and is independent of TPD1.

Keywords: Microsporogenesis, Tapetum, Cell wall, Cellulose, Sterility

Poster #224. LATERAL ORGAN BOUNDARIES DOMAIN (LBD)10 interacts with SIDECAR POLLEN/LBD27 to critically control pollen development in Arabidopsis (Submission 360)
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Flowering plants have the complex life cycle alternating between the multicellular diploid sporophyte generation and a haploid gametophyte generation. The haploid male gametophytes develop from unicellular microspores and produce the twin sperm cells that are necessary for double fertilization in flowering plant reproduction. During male gametophyte development, the diploid pollen mother cell undergoes meiosis to produce tetrad encapsulating tapetum cell. Development of the microspore involves microtubule-dependent migration of the nucleus to produce a highly polarized microspore, which undergoes an asymmetric mitotic division to form a bicellular pollen harbouring a larger vegetative nucleus and a smaller generative cell engulfed within the vegetative cell cytoplasm. The generative cell undergoes a symmetric second mitotic division, giving rise to a tricellular pollen with a vegetative nucleus and the twin sperm cells. Several components that play critical roles during male gametophyte development in Arabidopsis have been identified by genetic approach, shedding light on molecular mechanisms of pollen development. A member of LATERAL ORGAN BOUNDARIES DOMAIN (LBD) gene family encoding a class of plant transcription factors, SIDECAR POLLEN/LBD27, plays a key role in oriented division of polarized microspores for the asymmetric mitosis during Arabidopsis pollen development. Here we provide genetic evidence that LBD10 functionally interacts with LBD27 to critically control pollen development. Our results indicate that while LBD10 and LBD27 are differentially involved in male gametophyte development, genetic and protein-protein interactions between these two LBD proteins are crucial for Arabidopsis pollen development. This study was supported by grants from the Next-Generation BioGreen 21 Program (PJ00949103), Rural Development Administration and the Basic Science Research Program (grant no.
Keywords: lateral organ boundaries domain 10, male gametophyte development, mitotic division, pollen, microspore

**Poster #225. MOS7, an Arabidopsis Nup88 homolog Plays a Crucial role in Gametophytic and Zygotic Development.** (Submission 363)
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Nuclear pore complexes (NPCs) are multi-protein channel spanning the nuclear envelope and form a selective barrier between cytoplasm and nucleus of interphase cells. In plants and vertebrates, about 30 nucleoporins (Nups) are annotated and sharing similar domains. While the functions of Nups in animals are relatively well studied, the role of plants Nups and their regulation remains to be elucidated. Here, we show that MOS7 (Modifier Of Snc1,7), a human nucleoporin88 (Nup88) homolog in Arabidopsis plays a critical role in gametophytic and embryonic development. All experiments were carried out using MOS7/mos7-5 mutants because no homozygous mos7-5 mutants were obtained due to embryonic lethality. In the reproductive stage, mos7-5 heterozygous mutant showed abnormal nuclear behavior and aberrant cell division. Through microscopic analysis, we observed that mitotic spindle assembly, phragmoplast and cell plate formation were defective in MOS7/mos7-5 mutants during mega- and micro-gametogenesis. A yeast two hybrid screen and BiFC analysis identified dynein light chain and kinectin, two microtubule associated proteins (MAPs), as MOS7-interacting partners. During mitosis, MOS7 is localized at the mitotic spindle structure in proliferating cells. Overall, our data suggest that MOS7 plays an important role in microtubule dynamics together with MAPs during gametogenesis prior to fertilization and embryogenesis after fertilization.

Keywords: Nucleoporin, microtubule dynamics, gametogenesis

**Poster #226. Interplay between SWR1 and NuA4 chromatin remodeling complexes in the regulation of plant development in Arabidopsis** (Submission 372)
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The floral transition is a key developmental process in the lifecycle of plants and therefore is highly regulated. Chromatin remodeling plays an important role in the regulation of flowering time, and several chromatin-modifying activities have been shown to modulate the expression of master flowering genes, including the floral repressor FLOWERING LOCUS C (FLC). The SWR1 complex (SWR1-C) exchanges histone H2A by the histone variant H2A.Z in the FLC chromatin and this is required for the transcriptional activation of the gene. In yeast, SWR1-C shares four subunits with the NuA4 complex (NuA4-C). NuA4-C has histone acetyl transferase activity and is involved in the acetylation of histones H4, H2A and H2A.Z, suggesting that acetylation mediated by a putative Arabidopsis NuA4-C may also have a role in the regulation of flowering time. The S. cerevisiae NuA4-C is formed by 13 subunits, most of which present homologues in the Arabidopsis genome. It has been recently shown that the Arabidopsis homologues of the yeast Esa1 (AtHAM1 and AtHAM2) and Yaf9 (AtYAF9a) subunits regulate H4 acetylation levels at the FLC locus. We are interested in characterizing the function of the NuA4 homologues in Arabidopsis and the subunits shared with SWR1-C. We have isolated different mutant alleles for them and have generated double mutant combinations to uncover the possible existence of functional redundancy between homologues. The phenotypic analysis of these mutants has revealed that most of them show alterations in flowering time, as well as in the expression of master flowering genes. We aim to complete the functional characterization of these mutants to unveil the mechanism by which the interplay of NuA4-C and SWR1-C regulates flowering time in Arabidopsis.

Keywords: Chromatin remodeling, Plant Development, SWR1-C, NuA4-C
Poster #227. Embryonic and seedling phenotypes of csn2, a mutant for the COP9 signalosome (Submission 373)
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The COP9 signalosome (CSN) complex is involved in many aspects of plant life, including photomorphogenesis, hormone response and development of lateral organs. The complex is composed of eight subunits (CSN1 to CSN8). Null mutations in any subunit lead to the loss of the CSN holo-complex. csn mutants are seedling lethal, with a characteristic fusca phenotype (anthocyanin accumulation, small cotyledons, short roots). It has been suggested that csn seedlings arrest after germination as a consequence of not being able to progress past the G2 phase of the cell cycle, possibly due to the activation of DNA damage pathways (Dohmann et al., 2008, Development 135: 2013). However, csn mutants are able to complete embryogenesis and to germinate. We characterized csn2 (also known as fusca12) mutant embryos and seedlings. csn2 mutant seedlings have a smaller-than-normal root apical meristem (RAM) that gets consumed by six days after germination. Similarly, the shoot apical meristem ceases functioning after producing two true leaf primordia. A significant percentage of seedlings have phenotypes that could be auxin-related. We decided to trace the origins of these phenotypes during embryogenesis. csn2 embryos and endosperm develop at a slower rate compared to wild type siblings, indicating a slower cell cycle. Embryonic morphology is for the most part normal, and auxin transport appears unaffected. However, auxin response is reduced. There are also defects in the cells that generate the RAM: the hypophysis divides abnormally during the globular stage, leading to an abnormal RAM in which QC function is partially lost. Therefore, at least some of the seedling phenotypes have their genesis during embryo development, but are not fully expressed until after germination.

Keywords: Embryogenesis, CSN signalosome, Germination, Meristems

Poster #228. A mutant with helically twisted petal epidermal cells implicates cell wall polysaccharides in the control of conical petal cell morphology (Submission 375)
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The adaxial epidermal cells of Arabidopsis thaliana petals have a distinctive conical shape. Conical petal epidermal cells are present in most flowering plants, impart physical properties to petals such as color intensity and hydrophobicity, and are important for pollinator attraction in many species.
To identify genes required for the morphogenesis of conical petal epidermal cells, we screened a population of EMS-mutagenized plants for mutants with abnormally shaped petal epidermal cells. We isolated 17 mutants affecting at least 10 distinct genes with robust defects in petal epidermal cell morphology. Five mutants affecting four genes have flatter conical cells implying defects in cell expansion, while mutants in two distinct genes have the opposite defect of elongated conical cells. Two allelic mutations result in the base of the cones being narrow and may be involved in delimiting the subcellular region where anisotropic expansion occurs. Mutations in cyp77a6 and gpat6 exhibit abnormally cylindrical petal cells and loss of the cuticular nanoridges that cover floral organs, implicating the cuticle in control of cell shape. Most of the mutants display few or no defects in cellular morphology outside of petals, suggesting that the genes required to make a conical cell are distinct from those involved in morphogenesis of commonly studied cell types such as trichomes or pavement cells.
We have focused our studies on one mutant that affects cell expansion, which we have named dairy queen. dairy queen has defects in cell expansion with many conical cells failing to expand to the same extent as those of wild-type plants. The dairy queen conical petal cells also display a left-handed helical twist, causing the entire petal to be helically twisted. The dairy queen cell twisting is an anisotropic growth-dependent process; young isotropically expanding petal cells are not twisted, and then as petal cells expand anisotropically into a cone they spiral. The dairy queen defect is highly specific to conical petal epidermal cells with no cell expansion or spiral growth defects in other tissues. Preliminary molecular genetic analyses suggest that the dairy queen mutation affects a gene involved in the synthesis of pectin polysaccharides. We will present progress towards determining the effects of the dairy queen mutation on the cell walls of conical petals cells and how pectin might function to prevent helical growth of expanding cells.
Poster #229. BASIC PENTACYSTEINE proteins regulate the expression of a floral repressor FLOWERING LOCUS C (Submission 389)
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BASIC PENTACYSTEINE (BPC) proteins, which are named by their conserved five cysteine amino acids, regulate the expression of several genes in developmental pathway positively or negatively. In Arabidopsis, seven BPC proteins are widely expressed throughout the whole tissues and bind GAGA motif which potentially exists in the regulatory region over 3000 genes; however, in spite of their importance on plant development, functional mechanism has not been studied yet. To confirm whether a floral repressor FLOWERING LOCUS C (FLC) is a direct target of BPCs, we performed DNA motif analysis on the sequence of FLC gene and searched out six GAGA motifs in the target region. Because BPC proteins have functional redundancy, bpc pentuple mutant (bpc12346) was used as knock-out line for qRT-PCR analysis and FLC transcript level was lower compared to wild type; however, FLC expressed higher in the bpc7 mutant than wild type and this could be interpreted that BPC7 might have different function with transcript regulation because of its unique C-terminal region. Flowering time of bpc12346 and wild type was similar but bpc7 showed slightly late flowering phenotype than wild type under long day condition. FLC level of the overexpression lines decreased slowly by vernalization while the level decreased rapidly in the wild type FRI-Col, and therefore flowering time of BPC1 overexpression line was later than the wild type. Transcript levels of seven BPCs were also analyzed during vernalization but they showed similar expression level during cold treatment. Our results indicate that BPCs are new transcriptional regulator of FLC and this regulation might be conducted through the conformational change of chromatin structure.

Keywords: BPC, GAGA motif, FLC, Flowering, Vernalization

Poster #230. Screening upstream regulators of VERNALIZATION INSENSITIVE 3 in Arabidopsis thaliana (Submission 390)
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Vernalization, acquisition of competence for flowering accomplished by long term cold such as winter, is one of the most important mechanisms to determine the timing of flowering in plants. In Arabidopsis, vernalization is mediated by PRC2-mediated repression of strong floral repressor gene FLOWERING LOCUS C (FLC). PHD finger domain protein VERNALIZATION INSENSITIVE3 (VIN3) was reported as one of the key component in PRC2-mediated FLC repression. When VIN3 is absent, repression of FLC is not accomplished regardless of sufficient long term cold treatment. However, the question how long term cold signal can be perceived by plants is still elusive. Gradual increase of VIN3 transcript level in plants exposed to vernalization is well known, but how plants perceive long term cold is not known yet. Isolation of VIN3 upstream components may reveal how the VIN3 elevates gradually and how plant perceives vernalization signal. For this purpose, we generated a transgenic line with the minimal promoter of VIN3 fused to GUS reporter gene and performed EMS mutagenesis. Currently, we have isolated 3 vernalization hypersensitive mutants (p31, p102, and p773) which show increased expression of VIN3 in vernalization treatment. One of them, p31 shows callus-like phenotypes and sterile. The cloning of the corresponding genes is in progress.

Keywords: Flowering, Vernalization, VIN3, Screening

Poster #231. The role of ethylene action on CYP707A2 in seed germination (Submission 413)
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Germination is a process, occurring in the imbibed and non-dormant seed, in which the radicle breaks through the seed-covering layers to grow into a seedling. To activate this process, the seed must 'sense' multiple environmental cues and respond by reprogramming genome-wide transcriptional changes. One phytohormone -
Abscisic acid (ABA) - must be degraded, as it is a negative regulator. The total ABA content in the seed is highly regulated and controlled by a balance between its biosynthesis and degradation. CYP707A2, a member of the cytochrome P450 CYP707A family of ABA 8'-hydroxylases, is rapidly upregulated in imbibed seeds, acting as the primary ABA degradation enzyme during seed imbibition. It is not yet known how CYP707A2 expression is upregulated in response to environmental and hormonal signals, although ABI4 was shown to regulate CYP707A2 through direct binding. Ethylene, a gaseous molecule, plays an antagonistic role to ABA in seed germination control, and can modulate ABA sensitivity. Ethylene signaling mutants etr1 (ethylene response 1) and ein2 (ethylene insensitive 2) have increased ABA content and sensitivity, as well as increased dormancy and poor germination rates. To examine the role of ethylene in the regulation of CYP707A2, we used qPCR to analyze its expression in the seed in the ethylene signaling mutant ein2-44. We found that its expression is downregulated in imbibed ein2-44 seeds. In accordance with this, ABA measurements showed that the ABA content was higher when compared to Col-0. To visualize the CYP707A2 expression pattern and to quantify its expression in response to ethylene, a CYP707A2pro::CYP707A2-GUS line was constructed and is being analyzed. An understanding of how CYP707A2 is upregulated will contribute to our knowledge of how the imbibed seed initiates germination programs that stimulate or inhibit germination progression.

Keywords: seed germination, CYP707A2, ABA catabolism, ethylene

Poster #232. Uncoupling mid- and late embryogenesis phases by partial complementation of afl mutants in Arabidopsis

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ABSCISIC ACID INSENSITIVE3 (ABI3), FUSCA3 (FUS3), and LEAFY COTYLEDON2 (LEC2), collectively AFL, are master regulators of seed maturation. We examined the role of the AFL B3 DNA binding domain in the production of seed reserves in Arabidopsis thaliana. Quantitation of seed reserves and cytological observations of single, double and triple afl mutant embryos, revealed storage lipid and protein but not starch reserves to be positively and spatially regulated by AFL factors. ABI3 and FUS3 contribute to carbon partitioning since the abi3 mutation reduces seed protein content to a greater extent than lipid content, whereas fus3 has a more severe effect on triacylglycerol content. Furthermore, FUS3 exerts a greater control over fatty acid desaturation mediated by both FAD2 and FAD3, whereas ABI3 exerts a greater control over fatty acid elongation. Although ABI3 influences reserve content throughout the embryo, LEC2 and FUS3 act in distinct territories, the cotyledon and embryonic axis respectively. By analysing the ability of an individual ectopically expressed AFL to genetically suppress afl mutations, we show that the conserved B3 DNA binding domain is necessary and sufficient for the initiation of synthesis and accumulation of triacylglycerol and for the initiation, but not maintenance of seed storage protein synthesis, thereby demonstrating redundancy among the AFL and the existence of a threshold for function in the AFL pool. No individual AFL was able to suppress precocious germination nor initiate dormancy and tolerance to desiccation. Thus, mid- and late-embryogenesis programmes were uncoupled, consequently, reserve accumulation was prematurely terminated concomitant with viviparous development.

Keywords: Seed reserves, B3 domain transcription factor, triacylglycerol, Lipid, protein

Poster #233. Natural Accessions of Arabidopsis Differ in Sensitivity to a Loss of Chloroplast Translation

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Mutations that eliminate chloroplast translation in Arabidopsis result in embryo lethality. The stage of embryo
arrest, however, can be influenced by genetic background. To identify genes responsible for improved growth in the absence of chloroplast translation, we examined seedling responses of different Arabidopsis accessions on spectinomycin, an inhibitor of chloroplast translation, and crossed the most tolerant accessions with embryo-defective mutants disrupted in chloroplast ribosomal proteins generated in a sensitive background. The results indicate that tolerance is mediated by ACC2, a duplicated nuclear gene that targets homomeric acetyl-coA carboxylase (ACCase) to plastids, where the multi-domain protein can participate in fatty acid biosynthesis. In the presence of functional ACC2, tolerance is enhanced by a second locus that maps to chromosome 5 and heightened by additional genetic modifiers present in the most tolerant accessions. Notably, some of the most sensitive accessions contain nonsense mutations in ACC2, including the "Nossen" line used to generate several of the mutants studied here. Functional ACC2 protein is therefore not required for survival in natural environments, where heteromeric ACCase encoded in part by the chloroplast genome can function instead. This work highlights an interesting example of a tandem gene duplication in Arabidopsis, helps to explain the range of embryo phenotypes found in Arabidopsis mutants disrupted in essential chloroplast functions, addresses the nature of essential proteins encoded by the chloroplast genome, and underscores the value of using natural variation to study the relationship between chloroplast translation, plant metabolism, protein import, and plant development.

Keywords: Embryo-defective mutants, Chloroplast translation, Acetyl-coA carboxylase, Spectinomycin, Natural variation, Chloroplast protein import, Fatty acid biosynthesis, Tandem gene duplication, Essential chloroplast genes, Nonsense mutations

Poster/Talk #234. The developmental consequences of metabolic inhibition of polyamine biosynthesis (Submission 462)
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Methylthioadenosine nucleosidase (MTN) contributes to methionine regeneration by metabolizing methylthioadenosine (MTA), a common by-product of nicotianamine, ethylene, and polyamine biosynthesis. The Arabidopsis genome encodes two MTN isoforms, MTN1 and MTN2. Metabolic profiling of the Arabidopsis MTN-deficient mutant mtn1-1mtn2-1 revealed its higher MTA content, decreased nicotianamine and abnormal free polyamine profiles (including reduced spermidine and thermospermine). These observations are consistent with the hypothesis that polyamine and nicotianamine biosynthetic enzymes are sensitive to feedback inhibition by MTA in this mutant. As a result, mtn1-1mtn2-1 mutants have a complex pleiotropic phenotype including delayed development, male and female sterility and abnormalities in stem vasculature. Their stems have an increased number of vascular bundles, each with more phloem and xylem cells than stems in wild-type plants. This trait is similar to thick vein (tkv) a thermospermine synthase mutant. Both mutants also have defects in polar auxin transport. The phenotype of the MTN mutant is complemented by overexpression of SUPPRESSOR OF ACAULIS 51 (SAC51), a suppressor of the thermospermine-deficient mutant acl5-1. Delayed germination is the earliest phenotypic trait we have identified so far in mtn1-1mtn2-1 plants. We believe that the effects of MTA inhibition may be initiated even earlier in development. Thus, we are currently monitoring the expression of several fluorescent reporters for key metabolites or genes related to vascular development, in embryos of WT and MTN mutants. We are also investigating the requirement for MTN activity through tissue-specific complementation experiments. The results of these experiments will help to elucidate the role(s) of polyamines in vascular development.

Keywords: polyamine biosynthesis, vascular development, embryo development, spermidine, thermospermine, methylthioadenosine nucleosidase, sac 51

Emerging Topics

Poster #235. EXPO and Autophagosome in Arabidopsis: Biogenesis and Function (Submission 30)
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Using the Arabidopsis Exo70E2 (AtExo70E2) and its GFP fusion as tools, we have recently identified an unconventional secretion pathway that is mediated by a novel double membrane organelle termed EXPO (exocyst-positive organelle) in plants [1, 2]. We have also identified and characterized the exocyst complex and AtExo70E2 recruitment to EXPO in plant cells [3]. In addition, using BAR-domain protein SH3P2 and SH3P2-GFP as probes, we have recently illustrate the ER membrane origin and biogenesis of Autophagosome in Arabidopsis [4,5]. Current studies focus on understanding the underlying mechanism of EXPO biogenesis vs. Autophagosome formation as well as their possible relationship with PVC-vacuole function and protein trafficking in plants [6,7,8]. An update on this research program will be presented. Supported by grants from the Research Grants Council of Hong Kong (GRF, CUHK2/CRF11G, and AoE/M-05/12).

References:

Keywords: EXPO, Autophagosome, Organelle biogenesis, Organelle function

Poster/Talk #236. Roles of AtExo70E2 in exocyst recruitment, EXPO biogenesis and function, and plant growth and development (Submission 32)
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In contrast to a single copy of Exo70 in yeast and mammals, the Arabidopsis genome contains 23 paralogues of Exo70 (AtExo70). Using AtExo70E2 and its GFP fusion as probes, we identified a novel double-membrane organelle termed exocyst-positive organelle (EXPO) that mediatees an unconventional protein secretion in plant cells [1,2]. By performing transient expression in Arabidopsis protoplasts, we recently established the AtExo70E2-recruited exocyst subunit complex including Sec6 and Sec10 in plant cells [3]. Interestingly, AtExo70E2 is also capable of inducing EXPO formation in an animal cell line (HEK293A cells). Inversely, neither human nor yeast Exo70 homologues cause the formation of EXPO in Arabidopsis protoplasts. These results point to a specific and crucial role for AtExo70E2 in EXPO formation [3]. Current studies focus on investigating the possible roles of AtExo70E2 in regulating growth and development in Arabidopsis plants using both gain-of-function (overexpression) and loss-of-function (T-DNA insertional knock-out mutant) approaches, as well as the functional relationship between EXPO and Autophagosome [4,5,6] in Arabidopsis plants. Here, we will present an update on these studies. Supported by grants from Research Grants Council of Hong Kong (CUHK466011, 465112, 466313, CUHK2/CRF/11G, and AoE/M-05/12).

References:

Keywords: EXPO, Unconventional protein secretion, AtExo70E2, organelle biogenesis, autophagosome

Poster/Talk #237. Effects of aspirin and its metabolite on Arabidopsis thaliana (Submission 58)
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Increased consumption and subsequent excretion of pharmaceutical products by humans has led to an increased accumulation of these products in our environment. They are most commonly disposed of in our sewer systems, making their way into wastewater treatment plants and eventually into major bodies of water or the soil through biosolid applications. The focus of our study is to investigate the potential growth effects of these products on land plants as they have not been well documented. One of the first chemicals we selected for our study was aspirin. We applied various concentrations of aspirin (the active ingredient is acetylsalicylic acid or ASA) and its metabolite salicylic acid (SA) to multiple terrestrial plants at different growth stages to determine the effects of these compounds on various growth parameters, including germination rate, leaf growth, root growth, photosynthetic rates, and reproductive fitness. Preliminary results suggest that ASA and SA both negatively affect the germination rate and leaf growth at early stages of development of Arabidopsis thaliana when applied at 1 ppm or higher, and SA further reduces the height of mature Arabidopsis when its concentration is 10 ppm or above. Importantly, these concentrations are comparable to those found in the environment, with up to 1.5 ppm of ASA and 5.0 ppm of SA found in wastewater effluent samples in different areas of the United States. We are in the process of confirming the dose responses of these chemicals on Arabidopsis as well as on crop plants (e.g., corn and soy bean). The data will allow us to assess whether aspirin and its metabolite might impact the growth of multiple terrestrial plants, especially those that are economically important. Our current work is setting the foundation for future research that may result in changes in disposal of pharmaceutical products and common agricultural practices.

Keywords: pharmaceutical products, aspirin, acetylsalicylic acid and salicylic acid, growth and development

Poster/Talk #238. Keeping it similar: Interaction of HSP90 and miRNAs in the buffering of phenotypic variation in Arabidopsis thaliana (Submission 132)
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Robustness to genetic and environmental perturbations is a fundamental property of living organisms. Previous work has demonstrated the importance of the molecular chaperone HSP90 in maintaining organismal robustness. HSP90 inhibition reveals cryptic genetic and epigenetic variation with significant phenotypic consequences in flies, fish, plants, and yeast. The molecular mechanisms underlying the release of HSP90-dependent variation are not well understood. Recent studies found that microRNAs (miRNAs) are also important in maintaining robustness due to their function in modulating gene expression. In humans and flies, HSP90 is required for the formation of the miRNA-silencing complex. Moreover, in tobacco cell lines, HSP90 interacts physically with ARGONAUTE1 (AGO1), a key protein in the plant miRNA pathway, and a component of the miRNA-silencing complex. We hypothesize that HSP90 and miRNAs interact in maintaining organismal robustness in the plant A. thaliana. Using established assays for HSP90 function, we demonstrate that HSP90 and AGO1 interact genetically in the buffering of phenotypic variation in early seedlings. Moreover, AGO1 polymorphisms correlate with sensitivity to HSP90 inhibition of divergent A. thaliana strains, whereas polymorphisms in AGO10 and AGO5 do not. Using expression analysis we showed that the HSP90-AGO1 interaction in the buffering of variation is
complex. In addition, we tested several morphological traits and observed that both HSP90 and AGO1 are epistatic to each other in a trait-specific manner. Currently, we are comparing AGO1’s buffering potential and breadth to HSP90 by crossing ago1 mutants into different A. thaliana accessions. We present evidence that ago1 mutants buffer different genetic variations that HSP90.

Keywords: Buffering of phenotypic variation, Buffering of genetic variation, Phenotypic Robustness, AGO1, HSP90, Natural variation

Poster/Talk #239. Is trehalose 6-phosphate (T6P) a general signal gating developmental transitions? (Submission 157)
Vanessa Wahl\(^1\), Armin Schlereth\(^1\), Jathish Ponnu\(^2\), Magdalena Dzialo\(^1\), Markus Schmid\(^2\), Mark Stitt\(^1\)
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Plants integrate diverse environmental and endogenous signals to ensure the timely transitions between developmental stages. Trehalose 6-phosphate (T6P) has been implicated as a signal of sucrose status (1), thereby serving as a link between developmental processes and the metabolic and energy status of the plant. However, the molecular mechanisms by which this signal is integrated into the complex developmental networks are not well understood. T6P is synthesized from G6P and UDPG by TREHALOSE 6-PHOSPHATE SYNTHASE 1 (TPS1). A homozygous T-DNA insertion line is embryo lethal, with arrested embryos at the early torpedo stage, which is exactly when sucrose levels rise (2). TPS1 is expressed in vascular tissue throughout a plant’s life, but it also shows a distinct expression domain in the peripheral zone of the shoot apical meristem (SAM) from the heart stage of embryogenesis until right before the transition to flowering (3), where it obviously plays a role in cell cycle regulation and meristem maintenance. We found that T6P levels rise in the SAM over time, increasing more than two-fold during the floral transition in long day conditions as well as in short days. Interestingly, increasing TPS1 expression in the stem cells alone is sufficient to induce precocious flowering, while reducing T6P content by TREHALOSE 6-PHOSPHATE PHOSPHATASE (TPP) over-expression in the stem cells delays flowering (3). We have recently shown that T6P plays a central role in the induction of flowering acting, in the leaves via the FLOWERING LOCUS T node of the photoperiod pathway and at the SAM via the age pathway (3).

Recently it was shown that sugars, in particular glucose and sucrose, control the timing of the vegetative phase change by regulating the expression of MIR156A and MIR156C (4, 5). 35S:amiR-TPS1 plants with reduced levels of TPS1 and T6P are late flowering but are still able to set viable seeds. These plants have significantly more juvenile leaves, arguing for a delay in the vegetative phase change. The idea of T6P acting downstream of sucrose to regulate the vegetative phase transition is further supported by our transcript and genetic analyses. In summary, previous observations and our own data suggest that T6P is crucial for the correct timing of developmental transitions in general.

1 Lunn et al., Biochemical Journal, 2006
2 Eastmond et al., Plant Journal, 2002
3 Wahl et al., Science, 2013
4 Yu et al., eLife, 2013
5 Yang et al., eLife, 2013

Keywords: T6P signaling, flowering, meristem, vegetative phase change, developmental transitions

Poster #240. The Role of mRNA Alternative Polyadenylation in Root Cell Differentiation in Arabidopsis (Submission 224)
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Increasing evidence indicates that Alternative PolyAdenylation (APA) plays a significant role in gene expression regulation post-transcriptionally. The emerging theme is a broad impact of APA in cancers, immune- and neuronal-cell development in animals, and flowering time control, stress responses, disease resistance, and epigenetic regulations in plants. However, the impacts of APA in plant cell development and differentiation have
not been elucidated. Due to simple structure and a large collection of cell-type specific GFP-labeled transgenic plants, Arabidopsis root offers an excellent tractable system to study cell differentiation and cell-type specific gene expression. Using a fluorescence activated cell-sorting technology, each layer of cells in Arabidopsis primary root can be isolated. We have successfully generated poly(A) tag sequencing libraries for each cell type by a protocol we developed that specifically interrogates the junctions of 3'-UTRs and poly(A) tails (Wu et al, PNAS 108:12533, 2011; Thomas et al, Plant Cell 24:4376, 2012). Analysis of the large collection of the APA data indicated significant bias of APA profiles in different cell types, some of which could contribute to the specialization of each cell layer in the root. These APA are found to locate in different regions of the genes, including 5'-UTRs, coding sequences, introns, and 3'-UTRs; both in sense or antisense orientations. Many of them would have significant impacts on the transcripts affecting the functions of their encoding proteins. This is the first comprehensive analysis of APA in all cell-types of an organ in any organisms and reveals the potential role of mRNA 3'-end formation in cell differentiation and cellular identities.

Keywords: Cell differentiation, Gene expression regulation, mRNA processing, Alternative polyadenylation, Root development

Poster #241. A new paradigm for sustaining scientific resources (Submission 262)
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Research in plant biology increasingly relies on the use of online databases and data analysis tools. Most such resources receive grant funding for a limited period and then become stagnant or are discontinued. We have developed a new model for support of important resources capable of providing stable, scalable funding in proportion to the need for the resource. Our new model encourages responsiveness to researchers and long-term thinking about community needs. This new funding mechanism also increases the probability that valuable data can be preserved for the future. We have applied the new support model to the TAIR database (www.arabidopsis.org) and early results indicate that the model is capable of sustaining TAIR for the long term and will enable TAIR to continue to grow and develop at a rate that will keep pace with the evolving needs of the research community.

Keywords: TAIR, Databases, Tools, Funding, Sustainability

Poster #242. From Bench to Bountiful Harvests - Past, Present and Future Research Under the Umbrella of the Multinational Arabidopsis Steering Committee (MASC) (Submission 470)
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The Multinational Arabidopsis Steering Committee (MASC) has its origin in the 1990s when scientists from the United States, Europe, Japan and Australia formed an ad hoc committee to promote large-scale studies in Arabidopsis thaliana. During the last two decades the idea of a combined and coordinated effort accompanied by the policy of open data sharing has proven successful and led to the establishment of Arabidopsis thaliana as the reference plant. Currently, MASC is led by MASC chair Barry Pogson and co-chair Nicholas Provart with support of the MASC coordinator Luise Brand. MASC members work voluntary and the only funded member is the MASC coordinator, currently funded by the DFG. MASC subcommittees monitor and coordinate research in major Arabidopsis research areas: Bioinformatics, ORFeomics, Metabolomics, Natural Variation and Comparative Genomics, Phenomics, Proteomics as well as Systems and Synthetic Biology. The subcommittees comprise one of the three key groups of MASC alongside the major Arabidopsis projects and resources like ABRC, NASC, International Arabidopsis Informatics Consortium, TAIR and the new Arabidopsis Information Portal (www.araport.org). The third group of MASC is formed by, to date, 25 member countries each represented by local researchers. All MASC members contribute to the annual MASC report. This report outlines progress and activities of the Arabidopsis community as well as analysis and recommendations for the next year according to the road map (2012-2021) "From Bench to Bountiful Harvests" (Lavagi, Plant Cell, 2012). The MASC report 2013/2014 is published at the 25th International Conference on Arabidopsis Research (ICAR) and distributed to all attendees. The ICAR is a very successful initiative of MASC with support of the Arabidopsis community and is
hosted by the current MASC co-chair, who becomes MASC chair the following year. The next 26th ICAR will be on July 5th-9th 2015 in Paris, France.
If you like to know more about MASC please visit our new webpage: www.arabidopsisresearch.org or the web pages at TAIR: www.arabidopsis.org/portals/masc/index.jsp or directly contact us.

Keywords: Multinational Arabidopsis Steering Committee, road map 2012-2021, MASC report

Environmental Responses

Poster/Talk #243. Arabidopsis HPS10/ALS3 interacts with AtSTAR1 in tonoplasts to serve as a signaling hub for responses to P deficiency and Al toxicity (Submission 34)
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In many acid soils, phosphorus (P) deficiency and aluminum (Al) toxicity are the two most important constraints to plant growth and crop productivity. Although studies on plant responses to P deficiency have usually been conducted separately from those on plant responses to Al toxicity, it has long been observed that P and Al interact in affecting plant growth and development. Some evidence indicates that such interactions might result from the sharing of signalling components, but the molecular identity of such components is unknown. By analysing the Arabidopsis mutant hps10 (hypersensitive to Pi starvation10), we show that HPS10 encodes the previously characterized ALS3 (ALUMINUM SENSITIVE 3). HPS10 is an ABC (ATP-binding cassette) transporter-like protein that contains a transport domain but lacks an ATP-binding domain. In roots, HPS10 is specifically expressed in the vascular tissues; under P deficiency, however, its expression domain extends to all cell layers of roots. AtSTAR1 is also a transporter-like protein, but it contains only an ATP-binding domain and lacks a transport domain. The knockout mutant of atstar1 displays the same phenotype as hps10 in response to P deficiency and Al toxicity. Expression of both HPS10 and AtSTAR1 is induced by P deficiency. BiFC assays indicated that HPS10 interacts with AtSTAR1 in tonoplasts. Based on these results, we propose that HPS10/ALS3 and AtTSAR1 form a functional protein complex in tonoplasts to serves as a signaling hub to coordinate plant responses to P deficiency and Al toxicity.

Keywords: Phosphate deficiency, Aluminum toxicity, Signaling component, HPS10, ABC transporter

Poster #244. Diversity of Stomatal Responses to Different Environmental Conditions in Arabidopsis thaliana Ecotypes (Submission 50)
Sho Takahashi¹, Keina Monda¹, Juntaro Negi¹, Fumitaka Konishi¹, Shinobu Ishikawa¹, Mimi Hashimoto-Sugimoto¹, Nobuharu Goto², Koh Iba¹
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Stomata are small pores surrounded by guard cells that regulate gas exchange between plants and the atmosphere. Guard cells integrate multiple environmental stimuli and control the aperture width to ensure appropriate stomatal function for plant survival. Leaf temperature can be used as a convenient indicator of stomatal responses to environmental stimuli. In this study, 59 ecotypes that exhibited a low leaf temperature response rate to CO2 in leaf thermal imaging were selected from 378 Arabidopsis thaliana ecotypes. Measurement of stomatal conductance using a gas exchange system revealed particularly low stomatal response rates to CO2 changes in the three ecotypes of Koln (Kl-4), Gabelstein (Ga-0), and Chisdra (Chi-1). Stomatal responses to other environmental stimuli were also investigated. The stomatal response rate to light was similar to that to CO2, and generally was low. On the contrary, stomatal responses to humidity of these three ecotypes were not as low as that to CO2 and light. Interestingly, the response to abscisic acid, a plant hormone involved in the adaptation of plants to reduced water availability, was not entirely consistent with the response to humidity. This study demonstrates that the responses to CO2 and light share closely associated signaling mechanisms that are not generally correlated with humidity signaling pathways across the ecotypes. The results might reflect differences in the intrinsic response mechanisms to environmental stimuli between ecotypes.
Poster #245. Regulation of miR399f transcription by AtMYB2 affects phosphate-starvation responses in Arabidopsis (Submission 64)
Chan Min Kim¹, Dongwon Beak¹, Dae-Jin Yun¹
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Although a role for microRNA399 (miR399) in plant responses to phosphate (Pi) starvation has been indicated, the regulatory mechanism underlying miR399 gene expression is not clear. Here, we report that AtMYB2 functions as a direct transcriptional activator for miR399 in Arabidopsis Pi starvation signaling. Compared with untransformed control plants, transgenic plants constitutively overexpressing AtMYB2 showed increased miR399f expression and tissue Pi contents under high Pi growth and exhibited elevated expression of a subset of Pi starvation-induced genes. Pi starvation-induced root architectural changes were more exaggerated in AtMYB2-overexpressing transgenic plants compared with the wild type. AtMYB2 directly binds to a MYB-binding site in the miR399f promoter in vitro, as well as in vivo, and stimulates miR399f promoter activity in Arabidopsis protoplasts. Transcription of AtMYB2 itself is induced in response to Pi deficiency, and the tissue expression patterns of miR399f and AtMYB2 are similar. Both genes are expressed mainly in vascular tissues of cotyledons and in roots. Our results suggest that AtMYB2 regulates plant responses to Pi starvation by regulating the expression of the miR399 gene.

Keywords: microRNA399, plant responses to phosphate, AtMYB2

Talk #246. GI (GIGANTEA), a missing link between flowering and stress adaptation (Submission 65)
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Environmental challenges to plants typically entail retardation of vegetative growth and delay or cessation of flowering. We now report on a link between the flowering time regulator, GIGANTEA (GI), and adaptation to salt stress that is mechanistically based on GI degradation under saline conditions thus retarding flowering. GI, a switch in photoperiodicity and circadian clock control, and the SNF1-related protein kinase SOS2 functionally interact. In the absence of stress, the GI:SOS2 complex prevents SOS2-based activation of SOS1, the major plant Na+/H+-antiporter mediating adaptation to salinity. GI over-expressing, rapidly flowering, plants show enhanced salt sensitivity, whereas gi mutants exhibit enhanced salt tolerance and delayed flowering. Salt-induced degradation of GI confers salt tolerance by the release of the SOS2 kinase. The GI-SOS2 interaction introduces a higher order regulatory circuit that can explain in molecular terms the long observed connection between floral transition and adaptive environmental stress tolerance in Arabidopsis.

Keywords: adaptation to salt stress, GIGANTEA (GI), circadian clock control, SNF1-related protein kinase SOS2, Na+/H+-antiporter

Poster #247. Epigenetic switch of acetic acid synthesis confers plant drought tolerance (Submission 78)
Jong-Myong Kim¹, Taiko To², Motoaki Seki¹
¹RIKEN CSRS, Japan, ²NIG, Japan

Water deficit negatively impacts survival of organisms. To endure drought, plants trigger drastic changes in gene expression, cellular metabolism, and epigenetic information. However, how chromatin regulation affects these changes under water deficit stress, and which chromatin-modifying enzymes regulate stress tolerance, is rarely understood. Here we show that the histone deacetylase HDA6 epigenetically controls the acetate fermentation pathway; increases in acetic acid are essential for drought tolerance in Arabidopsis thaliana. While glycolysis is downregulated under drought stress, the acetate fermentation pathway genes PDC1 and ALDH2B7 are specifically activated. Notably, plants having mutations in these genes making them deficient in acetic acid biosynthesis show severe drought sensitivity. HDA6 directly binds to and represses those genes under normal conditions. HDA6 dissociates from these loci in response to drought stress, triggering increased histone acetylation, gene activation, and the accumulation of acetic acid. The hda6 mutation further enhances the upregulation of PDC1 and ALDH2B7 and the increase in endogenous acetic acid levels during drought, leading to...
substantial drought tolerance. Moreover, the external application of acetic acid can successfully enhance plant drought tolerance. Our findings indicate a novel strategy by which plants adapt to environmental deterioration by using an epigenetic switch of metabolic flow conversion.

Keywords: water deficit, abiotic stress, epigenetic, histone, metabolism

Poster #248. Search of the chemical compounds which alleviates thermal damage of photosynthetic electron-transport systems (Submission 79)
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Photosynthesis is often inhibited by high temperatures that limit plant growth and productivity. Both electron transport and rubisco activation have been suggested as the process responsible for the inhibition of photosynthesis caused by heat stress. However, attempts that prevent heat damage to photosynthetic machinery are still unsuccessful.

The RIKEN NPDepo chemical library contains about fourteen thousands pure compounds, half of which are natural products and their derivatives. With those chemical libraries, we try to identify the chemical compounds which suppress the damage of photosynthesis under high temperature treatment by using chlorophyll fluorescence as an indicator that is possible for a high-throughput chemical screening. Arabidopsis leaves exposed to heat treatment have shown a decline in the operating effective quantum yield of PSII and in the proportion of active PSI reaction centers. The establishment of the energy-dependent non-photochemical quenching was accelerated but its decline under illumination was inhibited. In first screening, we used the 80 of RIKEN NPDepo Authentic Library which contains a standard chemical compounds (m.w. 200-300, 10mg/ml) with bioactivity. Preliminary data show that chemical screening is useful for isolation of novel compounds that affect both moderate and severe heat stress. We found two candidates of "suppressor of Fv'/Fm' inhibition" and four candidates of "suppressor of NPQ induction". Compared with other compounds, the influence on these candidate compounds showed remarkable high by heat. Our goal is to find the chemical compounds and their target proteins that suppress the effects of heat stress mentioned above.

Keywords: heat, photosynthesis, chemical compounds

Talk #249. Characterization of the oxygen sensing mechanism in plants (Submission 80)
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In natural environments, plants often experience low oxygen conditions due to the occurrence of flooding events or high metabolic activity. In 2011, the oxygen sensing mechanism of plants was described (Licausi et al. 2011). In Arabidopsis thaliana, under normal oxygen conditions the ERF transcription factor RAP2.12 is localized at the plasma membrane via interaction with the Acyl-CoA binding proteins ACBP1/-2. Upon hypoxia, RAP2.12 relocalizes from the plasma membrane to the nucleus where it induces expression of hypoxia responsive genes. In addition, RAP2.12 stability is controlled via an oxygen dependent N-end rule pathway for proteasomal degradation. In the present study we further characterized RAP2.12 activity as key component of the oxygen sensing mechanism in Arabidopsis. We describe RAP2.12 relocalization and the induction of its target genes in a time- and oxygen-dependent manner. Our data reveal that reduction to 10% oxygen in air is sufficient to trigger RAP2.12 movement from the membrane into the nucleus, which correlates with the induction of hypoxia responsive genes. We also show that exposure of Arabidopsis plants to 1% hypoxia for 30 min is sufficient to trigger RAP212 the induction of its targets. When normoxic conditions are restored, RAP2.12 requires 3h before disappearing from the nucleus, which correlates with the hypoxia responsive genes returning to normoxic levels suggesting the existence of active reduction of transcriptional regulation to stop the anaerobic response. By using fusions between RAP2.12 with a photoconvertible fluorescent protein (mEOS) we could discern the contribution of relocalization from de novo protein synthesis to the nuclear accumulation of RAP2.12. Taken together, we were able to characterize in detail the contribution of intracellular RAP2.12 relocalization to the oxygen sensing mechanism in plants.

Keywords: hypoxia, N-end rule pathway, ERF VII family, low oxygen response, mEOS

Poster/Talk #250. Regulatory network models of drought responses in Arabidopsis thaliana (Submission 110)
Ulrike Bechtold¹, Vicky Buchanan Wollaston², Jim Beynon², Katherine Denby², David Wild², David Rand², Nicholas Smirnoff³, Philip Mullineaux⁴
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Water availability is the biggest single limitation on crop productivity worldwide. The improvement of plant productivity while reducing agricultural water use is an urgent objective to sustain or improve production while conserving natural resources. The complex mechanisms, which coordinate adjustments to drought stress are generally supported by changes in gene expression. Identification of genes that promote drought tolerance is therefore important for the improvement of crops. It is therefore important to understand the complex regulatory networks of how plants respond to water limitation throughout development in order to maintain crop performance. We are using a couple of approaches to identify novel genes responsible for altering lifetime We have produced a high-resolution time series microarray dataset and coupled with network modelling approaches try to understand the temporal coordination of drought stress at the transcriptional level. We have performed a slow drying experiment of soil-grown Arabidopsis plants and analysed metabolic and physiological responses and transcriptional changes over a 14-day period. A total of 1825 differentially expressed genes (DEGs) were identified. Functional annotation noticeably lacked gene ontology (GO) terms associated with drought, water deprivation or abscisic acid (ABA), but showed a significant enrichment for carbohydrate metabolism, suggesting an early osmotic adjustment and sugar signaling in response to drying. Changes in gene expression were only observed markedly after physiological adjustments, coinciding with a drop in carbon assimilation and increases in ABA during the latter half of the experiment. This suggests that early physiological responses to a decline in soil water are not driven by major changes in gene expression or hormonal changes. Using a Bayesian state space modelling approach we identified highly connected genes (hubs), and mutants of hub genes have altered responses to drought stress and growth phenotypes, and are believed to be involved in sugar signalling and plant development.

Keywords: drought stress, transcriptional profiling, network modelling

Poster #251. Germinability as a “reader” of heterologous gene effects in early responses to abiotic stress (Submission 129)
Michael Schlappi¹, Marian Lund²
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The plant specific class of hybrid proline- or glycine-rich proteins (HyP/GRP) are putative cell-wall/plasma membrane associated proteins. Their coding regions form gene families, and even small plant genomes such as Arabidopsis thaliana contain approximately 30 HyP/GRP genes. The mature proteins have two distinct domains: a hydrophilic proline-rich or glycine-rich repetitive domain (PRD or GRP, respectively) at the N-terminus, and a hydrophobic domain with eight cysteine residues in a specific order called the eight-cysteine motif (8CM) at the C-terminus. The unprocessed proteins contain putative signal peptides at the immediate N-terminus for presumed secretion into the cell wall and/or cell membrane. While they share similarity with lipid transfer proteins and protease inhibitors, the specific cysteine spacing and hydrophobicity of the C-terminus makes them a distinct class of proteins. This poster summarizes recent data from our lab using Arabidopsis as a “reader” of heterologous HyP/GRP gene effects in response to abiotic stress, and hypothetically addresses numerous unanswered questions that could be explored to understand the role this gene family during plant growth and development.
Poster/Talk #252. GENETIC ENGINEERING OF ABIOTIC STRESS RESPONSE AND PLANT GROWTH IN ARABIDOPSIS (Submission 151)
Yuriko Osakabe¹, Kazuo Shinozaki¹
¹RIKEN CSRS, Japan

To overcome adverse effects of abiotic stresses, plants develop a wide-range of adaptive mechanisms, including growth adjustment and stress tolerance via various factors involved in the phytohormone signaling, membrane transport, and gene transcription. Several members in ion transport systems, such as SLAC1, GORK, OST2, and KUP play important roles in stomatal responses. The phosphorylation and activation of the transport systems and transcription factors have also important roles in the control of stress response. We have generated Arabidopsis multiple mutants for KUP6, KUP8, KUP2/SHY3, and GORK, namely kup268 and kup68gork, that exhibited enhanced cell expansion and decreased stress tolerance, suggesting the redundant functions of these transport systems in osmotic adjustment to water deficit stress. The direct control of ion transport systems by ABA signaling factors has been shown, in which ABA-activated SNF1-related protein kinase 2E (SRK2E) phosphorylates the cytosolic regions of transporters. To improve the stress tolerance in Arabidopsis, we carried out the genetic engineering of such transporters and signaling factors by genome editing tools, which act as key factors in responses to the abiotic stresses. We will discuss the further applications of the genome engineering of model and crop plants by using new technologies for genome editing.

Keywords: abiotic stress, phytohormone, phosphorylation, transport systems, genetic engineering, genome engineering, water deficit stress

Poster/Talk #253. How and Why Plants 'Memorize' their Experience of Heat Stress (Submission 152)
Yee-Yung Charng¹, Siou-Ying Lin¹, Yu-Ting Juan¹
¹Academia Sinica, Taiwan

In the natural environment plants are frequently exposed to high temperatures that cause heat stress (HS). Exposure to less severe HS increases tolerance to subsequent severe HS, a phenomenon known as priming or acclimation. Despite not having a nervous system like animals, emerging evidence suggests that plants can transiently 'memorize' their experience of HS and preserve the acclimation effect for a few days. Our previous studies suggested that at least two mechanisms at the transcriptional and posttranscriptional levels maintain the HS memory in Arabidopsis. One mechanism involves a transcription cascade mediated by HSFA1s and HSFA2 that prolongs the transcriptional activities of the HS response (HSR) genes. The other mechanism involves a positive feedback loop between two heat-inducible proteins HSP101 and HSA32; HSP101 enhances the translation of HSA32 during recovery after heat treatment, and HSA32 retards the decay of HSP101. Our recent data show that the feedback loop between HSP101 and HSA32 also works in rice to maintain HS memory. However, disparity in maintaining HS memory was observed between indica and japonica rice cultivars, suggesting that HS memory has a role in plant adaptation to different environments. Here, we report our work on HS memory mediated by transcription factors HSFA1s and HSFA2 in Arabidopsis in greater detail. HSFA2 is a heat-inducible gene under the strict control of the HSFA1s. Microarray analysis showed that a subset of genes (about 100 genes) of the Arabidopsis genome are significantly affected (more than 2-fold change) by repeated HS treatment owing to prior HS (37 degree C, 1 h), a phenomenon called transcription memory. The transcription memory of two of these genes, ASCORBATE PEROXIDASE2 (APX2) and MYO-INOSITOL PHOSPHATE SYNTHASE2 was confirmed by quantitative RT-PCR and was shown to last for at least 5 days. HSFA2 is required for transcription memory, but is not sufficient in the absence of HSFA1s. Transcription memory is not simply due to a higher HSFA2 protein level induced by prior HS. Transgenic plants expressing luciferase gene fused to APX2 promoter recapitulated transcription memory suggesting that cis-elements are involved. Given that HSFA2 is also involved in response to high light and hypoxia stresses, the HS memory conferred by the HSFs is not only beneficial in tolerance to repeated HS but also provides cross protection against other stresses.

Keywords: heat stress, heat acclimation, stress memory, heat-shock factor
Poster #254. Molecular dissection of the Arabidopsis phosphate exporter PHO1 reveals three domains fulfilling distinct functions in subcellular localization, phosphate transport and response to phosphate deficiency (Submission 153)

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The Arabidopsis PHO1 is the prototypical phosphate (Pi) exporter, with homologues present in fungi and animals. In plants, PHO1 is primarily involved in the loading of Pi into the root xylem, and is thus essential for Pi transfer from roots to shoots. Consequently, the pho1 mutant shows low shoot Pi content. PHO1 has also been implicated in Pi signaling, impacting the response of shoot growth to Pi deficiency. While the role of PHO1 in Pi homeostasis is well described, the structural basis for its functions is unknown and the role of PHO1 homologues in Arabidopsis and other species is less investigated. Yet, all PHO1-like proteins share the same structural organization. They are composed of a hydrophilic N-terminal SPX domain, followed by 4 alpha-helices and a hydrophobic C-terminal EXS domain. While SPX and EXS domains are found in several eukaryotic proteins, their role is essentially unknown, although the SPX domain is found in several proteins involved in various aspects of Pi homeostasis. The precise topology of PHO1’s association with the membrane, and in particular of the EXS domain, was determined using a combination of redox-sensitive GFP and bi-molecular fluorescence complementation. Expression of PHO1 variants containing various combinations of domains in both tobacco transient assays and complementation of the Arabidopsis pho1 mutant has revealed that the 4 alpha-helices and EXS domain are the minimal regions essential for Pi export, but that the SPX domain is essential for proper pho1 complementation in Arabidopsis. The EXS domain was found to have an essential role in targeting PHO1 to the Golgi/TGN compartment. Surprisingly, expression of only the EXS domain in the pho1 mutant resulted in improvement of shoot growth despite low shoot Pi content. These results uncover a new role for the EXS domain of PHO1 in modulating the response of plants to Pi deficiency and uncoupling shoot growth response from Pi content.

Keywords: phosphate transport, phosphate deficiency response, PHO1

Poster #255. Understanding Molecular Interactions Controlling Iron Homeostasis in Plants (Submission 223)

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Iron (Fe) is an essential nutrient for the growth and development of plants and animals. As plants are major dietary source of Fe, understanding Fe homeostasis in plants is important for improving crop yields, and human and animal nutrition. Engineering plants that maintain Fe homeostasis under conditions of fluctuating nutrient availability requires understanding the molecular mechanisms underlying Fe uptake from soil, transportation and storage, especially under Fe-deficient growth conditions. How, exactly plants sense Fe-deficiency before imparting any response is yet to be elucidated. In our study, we identified two novel co-regulated proteins playing important roles in Fe homeostasis; bHLH transcription factor POPEYE (PYE) and BRUTUS (BTS). BTS contains three putative Fe-binding hemerythrin (HHE) domains, a CHY-type zinc finger domain and a RING (E3 ligase) domain. PYE directly regulates many Fe homeostasis genes such as FRO3, NAS4 and ZIF1. Both BTS and PYE expressions are tightly co-regulated in the root vasculature under Fe-deficiency. Disruption of PYE function leads to poor growth response while a mutation in BTS leads to increased tolerance under Fe-deficient conditions. Protein-protein interaction analysis revealed no interaction between BTS and PYE, but both interact with three PYE-like bHLH-transcription factors (PYEL), ILR3, bHLH104 and bHLH115. BHLH proteins commonly forms hetero-dimers and/or homo-dimers to regulate target genes. BTS interacts with all three PYEL and bind with Fe, suggesting it may act as iron sensor that regulates iron-responsive bHLH transcription factors under Fe-deficient conditions in roots. Our study indicates that BTS represses the iron deficiency response in the absence of iron by controlling these bHLH proteins.

Keywords: Iron-deficiency, Iron-homeostasis, BRUTUS, Hemerythrin, POPYE
Poster #256. Ethanol-Induced Autophagy Is Required for Regulation of Ethylene-Mediated Hypoxia Signaling in Arabidopsis (Submission 237)
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¹Sun Yat-sen University, China

Plant autophagy is a conserved recycling system for massive degradation of intracellular components. Previous studies in Arabidopsis suggested that it can be stimulated by various environmental stresses including starvation, salt, drought, oxidative stress, and pathogen infection. Here, we report the requirement of autophagy in regulation of plant response to submergence, an abiotic stress leading to hypoxia in plant cells. The transcripts of Arabidopsis autophagy-related genes (ATGs) and the autophagosome formation are induced upon submergence. The atg mutants display hypersensitivities to hypoxic stress and treatment with ethanol, which phenotypes are dependent on an intact salicylic acid signaling. Moreover, metabolic profiling reveals that the leaf starch degradation and ethanolic fermentation are elevated in the submerged atg mutants. Further, exogenous ethanol application activates autophagy in the GFP-ATG8e transgenic lines and promotes accumulation of reactive oxygen species (ROS) in the atg mutants. Observations that phenotypes of atg mutants are rescued by an ethylene-overproducer eto1 and that autophagy is activated by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), suggest a novel feedback regulatory loop of ethylene to reinforce autophagy. These findings demonstrate that the ethanol-induced autophagy is a protective process in plant response to hypoxia by modulating the homeostasis of ROS and ethylene signaling in Arabidopsis.

Keywords: autophagy, ethanol, ethylene, hypoxia, reactive oxygen species

Poster #257. Characterization of the MybD transcription factor involved in the regulation of anthocyanins biosynthesis in Arabidopsis (Submission 259)
Nguyen Hoai Nguyen¹, Hojoung Lee¹
¹Korea University, Korea, The Republic of

Myb transcription factors are responsible for many signaling pathways in plant system. For example, several Myb transcription factors play important roles in the regulation of the critical steps of flavonoids biosynthesis. The accumulations of anthocyanins are very sensitive to endogenous as well as exogenous cues such as light, abiotic stresses and various hormones. In this study, we found that the mybd ko mutants accumulate less anthocyanins than the wild type, while the MybD overexpressor lines have increased levels of anthocyanins than the wild type. The MybD is induced by cytokinins whereas its' transcript levels are reduced in dark condition. The transcript levels of genes for anthocyanins biosynthesis such as DFR, LDOX and PAP1 were increased in the MybD overexpressor lines in comparison to those of the wild-type. Taken together, the MYBD is a novel transcription factor which can work as a cross talk component of the light and cytokinin signaling, leading to the regulation of anthocyanins biosynthesis in Arabidopsis.

Acknowledgements
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Keywords: anthocyanins, Myb transcription factor, cytokinin, light, environmental stress

Poster #258. The Role of F-box-proteins in Stress-related Signaling in Arabidopsis thaliana (Submission 266)
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Induced defenses and stress responses play a major role in plant disease resistance and are regulated by a network of interconnected signal transduction pathways with the plant hormones ethylene, jasmonic acid and salicylic acid as crucial mediators. These specific hormone-mediated signaling cascades trigger distinct sets of stress-related genes leading to tolerance or resistance. F-box proteins (roughly 700 in Arabidopsis) are important components in these stress responses. They act as members of SCF protein complexes, which target substrate proteins for modification and degradation and thereby allow plants to respond rapidly to environmental stress.
factors. They are involved in various processes including plant metabolism, hormonal responses as well as responses to environmental stresses. For example Arabidopsis thaliana F-BOX protein MORE AXILLARY BRANCHING 2 (MAX2) has previously been characterized for its role in plant development. MAX2 has been indicated essential for the perception of the newly characterized phytohormone strigolactone, a negative regulator of polar auxin transport in Arabidopsis. Recently MAX2 was also shown to influence stomatal closure, demonstrated as decreased tolerance to drought stress. Also several other F-box proteins (e.g. TIR1, AFB4, COI1) have been shown to be involved both in plant development and/or stress responses. The objective of this study is to identify and characterize specific F-box proteins involved in stress signaling and components interacting with these F-box proteins. F-box proteins of Arabidopsis are organized into a superfamily (Gagne et al. 2002) out of which subfamilies C1-C4 are known to contain many proteins associated with stress signaling. To elucidate the function of F-box proteins belonging to subfamily C3 in the regulation of stress responses, we employ a reverse genetic screening approach and characterize T-DNA mutant lines of this subfamily of F-box proteins for their performance under abiotic (cold, ozone, drought) and biotic (bacterial pathogens Pectobacterium carotovorum and Pseudomonas syringae) stresses.

Keywords: Arabidopsis, F-box proteins, Pathogen responses

Poster/Talk #259. Asymmetric Unproductive Alternative Splicing Mediates Responses of the Central Circadian Oscillator to Environmental Stresses and Pathogen Infection (Submission 267)
Sergei Filichkin, Jason Cumbie, Palitha Dharmawadhana, Pankaj Jaiswal, Jeff Chang, Saiprasad Palusa, A.S.N. Reddy, Molly Megraw, Todd Mockler
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Alternative splicing frequently generates nonsense transcripts harboring premature termination codons (PTCs) in Arabidopsis circadian genes. Daily oscillations of the circadian transcripts that contain PTCs are synchronized with functional mRNAs under physiologically optimal conditions. Under physiologically extreme thermocycles or upon infection with P. syringae, oscillations of PTC-containing isoforms of the CIRCADIAN CLOCK ASSOCIATED 1 (CCA1) mRNA peaked at different times of day compared to pre-mRNA and functional mRNA. PTC+ CCA1 mRNA accumulates in the nucleus, escapes nonsense mediated mRNA decay and specifically binds to the splicing factor SR45 via retained intron. General model of asymmetric, unproductive alternative splicing implies that cyclical abundance of the functional mRNAs is regulated through switching splicing machinery toward nonsense transcripts. The CCA1-specific model predicts that robust oscillations of the CCA1 functional mRNA under environmental stress is regulated by a compensatory production and reversible sequestration of out-of-phase PTC+ intron retaining intermediates. Our results suggest that similarities in transcriptomes of plants with mutations in the nonsense mediated decay pathway and environmentally stressed plants are likely to be caused by disequilibrium of nonsense and functional transcripts.

Keywords: Alternative splicing, intron retention, circadian clock oscillator, Arabidopsis, circadian clock, nonsense mediated mRNA decay, environmental stress CCA1, Pseudomonas

Poster #260. Functional characterization of RCI8, a NAC transcription factor involved in Arabidopsis response to low temperature (Submission 296)
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Cold stress adversely affects plant growth and development, and limits crop distribution and yield. Diverse plant species tolerate cold stress to a varying degree. Plants from temperate regions have evolved an adaptive response, known as cold acclimation, whereby they are able to increase their freezing tolerance after exposure to low-nonfreezing temperatures. During cold acclimation, plants modify their metabolism and growth to adapt to freezing temperatures mainly by reprogramming gene expression. The identification of molecular mechanisms that control these changes in gene expression is crucial to understand how plants integrate responses to unfavourable environments to develop and reproduce correctly, and has the biotechnological potential to generate molecular tools to improve crop tolerance to abiotic stresses. We have identified a new gene of Arabidopsis
whose expression is induced by low temperature at low/medium levels. This gene, named Rare Cold Inducible (RCI) 8, encodes a NAC transcription factor. Our results indicate that the cold induction of RCI8 is mediated by the CBFs. The physiological characterization of rci8 mutants revealed that they have a decreased tolerance to freezing temperatures before and after cold acclimation. Conversely, Arabidopsis transgenic plants overexpressing RCI8 showed increased constitutive freezing tolerance and cold acclimation capacity. Gene expression analysis in rci8 mutant plants exposed to low temperature showed that RCI8 regulates the expression of a number of genes reported to be involved in freezing tolerance. All these data evidence that RCI8 functions as a positive regulator of freezing tolerance in Arabidopsis by controlling cold-induced gene expression.

Keywords: Cold acclimation, Cold response, Freezing tolerance, Abiotic stress

Poster #261. Water loss sensitivity varies with the difference of slac1 promoter region between Col and Ws of Arabidopsis thaliana (Submission 307)

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To understand how plants coped with dry stress, we investigated dry stress tolerance of two Arabidopsis thaliana accessions, Columbia-0 (Col-0) and Wassilewskija-2 (Ws-2). The experiment was performed by detaching the aerial-parts of leaves and was followed by weighing them every 10 minutes up to 60 minutes. It was resulted that the Col-0 showed higher dry stress tolerance than the Ws-2.

To identify the gene(s) that made the difference of their dry stress tolerances, we next performed a QTL analysis using recombinant inbred lines (RILs) derived from crossing between the Col-0 and the Ws-2. The analysis resulted in indicating a QTL with the highest LOD score of over 3 on chromosome 1. Surveying around the QTL region, we extrapolated that a gene named SLAC1, which was known to be associated to stomatal closure, was likely responsible for the difference of the tolerance between the two accessions. We compared the amino acid sequences of the SLAC1s. We found few functional differences between the Col-0 and the Ws-2 although we noticed that the Ws-2 lacked a part of the upstream region from -376 to -2804 of the Col-0 SLAC1 sequence, which included a gene (At1g12490).

Transcript levels of SLAC1 gene in stomata and mesophyll of Col-0 and Ws-2 were then determined by quantitative-PCR analysis. Slac1 expressed higher in stomata of Col-0 than in those of Ws-2, while the expression level showed no difference between the Col-0 and the Ws-2 in mesophyll. We subsequently performed histochemical GUS staining analysis using transgenic plants that were transformed into Col-0 or Ws-2 SLAC1 promoter::GUS constructs. The results revealed that SLAC1s were mainly localized at stomata in Col-0 whereas they were at leaf veins in Ws-2.

We concluded that the promoter region of Col-0 had a higher potential to express SLAC1 at stomata in comparison to that of Ws-2. Consequently, the Col-0 was able to close their stomata more quickly than the Ws-2 so that the Col-0 had better dry stress tolerance than the Ws-2.

Keywords: dry stress tolerance, stomata, slac1

Poster #262. Elucidating the Role of Symplastic Signaling in the Phosphate Starvation Response (Submission 324)

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The ability of a plant to respond to changes in its environment is important to its development and survival. Under phosphate starvation, one important foraging tactic is differential growth: allocating resources for growth of roots in nutrient-rich soils areas and ceasing growth of roots in less fertile soil. Since differential growth requires that roots not only "know" the quality of their soil, but also the quality of their soil relative to other roots on the plant, we suspect that cell-cell communication in the form of symplastic signaling is important for an integrated response to nutrient availability and that some cell layers in the root may serve as master regulators or integrators of the nutrient response. We are testing which cell layers contribute most to the stress response by inhibiting symplastic signaling between specific tissues in Arabidopsis roots. The root response is being assessed using standard microscopy to assess visible phenotypes and fluorescence activated cell sorting (to individually isolate specific
cell layers in the root) followed by transcriptional profiling. These experiments will allow us to determine the genes involved in the ability of the root to regulate differential growth under phosphate stress and how tissue-specific blocks to cell-to-cell signaling affect developmental and genetic plasticity.

Keywords: Symplastic signaling, Phosphate stress, Abiotic stress

**Poster #263. Role of predicted RAD4 interacting proteins in Arabidopsis thaliana UV damaged DNA repair** (Submission 340)
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Ultraviolet (UV) radiation poses a threat to the integrity of genomic DNA. Cells have inherent repair mechanisms to identify and repair UV damaged DNA. The light independent DNA repair pathway nucleotide excision repair (NER) is similar in plants, humans and yeast. NER of untranscribed DNA is referred to as Global Genomic NER (GG-NER). Deficiencies in NER result in serious disorders, for instance mutation of the human nucleotide excision repair gene XPC can cause the serious disease Xeroderma pigmentosum. The yeast (Saccharomyces cerevisiae) homologue of human XPC is RAD4 (Radiation sensitive 4), and the Arabidopsis homologue is AtRAD4. The Arabidopsis Interaction Viewer (AIV) predicts, based on homology, that AtRAD4 will interact with Arabidopsis homologues of other known NER components such as RAD23 and XPB. In addition, the AIV predicts high confidence interactions between AtRAD4 and novel RNI (Ribonuclease Inhibitor) -like superfamily proteins which we to refer to as RNI1 and RNI2. Another unknown RNI-like superfamily protein, RNI3, was found to be the closest homologue of RNI1. In our current research, we are studying the role of these predicted RAD4 interacting proteins (RNI 1,2,3) in UV damaged DNA repair. Our objectives are to generate loss of function and gain of function lines for the predicted interacting genes followed by UV tolerance assays. We will also examine the interaction between RAD4 and the predicted interacting proteins as well as the interaction among the RNI proteins. Our progress will be reported.

Keywords: UV damaged DNA repair, Global Genomic Nucleotide excision repair (GG-NER), RAD4

**Poster #264. The Role of Peroxisomal Metabolism in Modulating Photosynthesis** (Submission 347)
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Plant peroxisomes are linked to chloroplasts by a number of metabolic pathways, yet how peroxisomal metabolism impacts photosynthesis is unclear. To this end, we took a reverse genetic approach by screening Arabidopsis mutants for genes encoding peroxisomal proteins for deficiencies in photosynthesis. Preliminary results indicated that both photorespiration pathway and NADH/NADPH homeostasis play important roles in regulating photosynthesis. Consistent with previous studies, mutants of genes involved in photorespiration showed significantly decreased PSII quantum yield efficiency (phi2), increased Non-Photochemical Quenching (NPQ) and elevated reactive oxygen species (ROS) under high light. Besides, mutants of genes that are involved in NADH/NADPH generation exhibited altered phi2 and NPQ, which suggested that peroxisomal NADH/NADPH production may have a critical role in regulating photosynthesis. We propose that the photorespiratory metabolites in peroxisomes may modulate the function of key components in photosynthesis and photo-protection, and NADH/NADPH can impact photosynthesis indirectly through interacting with photorespiration or directly via regulating redox state required in photosynthesis. Overall, our results strongly suggest that peroxisomal metabolism is highly important for photosynthesis and photo-protection.

Keywords: Peroxisome, Photosynthesis,Photorespiration

**Poster #265. A physiological and gene expression approach to the relationship between multiple stresses in Arabidopsis thaliana and its close relatives in the genus Boechera** (Submission 350)
Genna Gallas¹, Sarah D’Antonio¹, Elizabeth Waters¹
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We are studying abiotic stress responses in Arabidopsis thaliana and five species in the genus Boechera, which
are found in a variety of environments. Many Arabidopsis genomic resources can be utilized for Boechera because of their close taxonomic relationship. Previous work has shown that A. thaliana and Boechera species have different tolerances to heat stress. Here we tested the impacts of high light and combined stress of heat and high light. Stress treatments include basal (direct impact) and acquired (plants pretreated with less intense stress). Plants were grown at 22degC and 150uE for 7-10 days. Heat stress treatments were for 3 hours at 38-45degC. High light treatments were at 1500uE for 3 hours. The combined treatments were both heat at 38-45degC and high light at 1500uE. Acquired pretreatments included pretreatments of 38degC and 38degC/1500uE, followed by 3 hour stress treatments from 41C-45degC, at both 150uE and 1500uE. For gene expression analyses, plant tissue samples were collected directly following stress treatments. Plant responses to the stress treatments were quantified via: 1) whole-organism response, 2) photosynthetic and 3) gene expression. The organismal response indicates the amount of cell death that occurred. The photosynthetic response was measured by Pulse-Amplitude-Modulation as chlorophyll fluorescence, a measure of plant photosystem II activity. Gene expression was quantified by measuring the changes in expression of stress-induced genes using quantitative real-time PCR.

We found that high light alone had a small but significant impact, and there is a strong effect on both photosynthesis and plant growth when high light and heat are combined. The Boechera species had significantly different responses to stress than did Arabidopsis thaliana. In addition, our results revealed a surprising level of variation among Boechera species in their abiotic stress responses, despite their close evolutionary relationship. A combined stress pretreatment gives photosynthetic protection against single and combined stresses in some Boechera species but not to others. The species show different patterns of gene expression, notably some stress-induced genes were expressed at much higher levels in A. thaliana than in Boechera. Because the Boechera species have higher tolerance to stresses than A. thaliana, they are ideal for studying the evolution of stress tolerance and for understanding how plants adapt to combinations of stressors.

Keywords: Chlorophyll fluorescence, Gene expression, Heat stress, Light stress, QPCR, RT-PCR, Relatives of Arabidopsis, Abiotic stress, Ascorbate peroxidase, Heat shock protein

**Poster #266. Environmental Responses of An Arabidopsis Ecotype, Me-0, with Giant Stomata** (Submission 359)
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Stomata are the morphological structures that control gas exchange and transpiration in plants. In general, there is an inverse relationship between stoma number and size, characteristics which vary greatly among species and genotypes. Changes in stomatal density and size can affect stomatal conductance, and thereby carbon assimilation, because photosynthesis and stomatal conductance are closely related. Arabidopsis thaliana has been collected from a wide range of habitats distributed primarily over the northern hemisphere. Genetic differences between local populations (ecotypes) presumably are associated with adaptations to the prevailing environmental conditions. We analyzed approximately 400 Arabidopsis ecotypes for CO2-induced stomatal responses, and found that the Me-0 ecotype shows particularly high stomatal conductance and very large stomata. We studied Me-0 cells by flow cytometry and image analyses and found that Me-0 guard cells are t tetraploid. This fits general observations of enhanced cell expansion caused by polyploidy. Furthermore, microarray analyses showed high expression levels of genes related to cell wall modification, which may induce expansion of the guard cells. To clarify the influence of large stomata on stomatal responses, we compared Me-0 with artificially induced tetraploid Col, which possesses large stomata like Me-0. Interestingly, tetraploid Col did not exhibit high stomatal conductance due to small stomatal aperture, whereas Me-0 showed large stomatal aperture because of increased levels of malate2- and K+ in guard cells. These results implied the advantageous effects on gas exchange of the Me-0 phenotype are not necessary repeated in artificial tetraploid plants.

Keywords: stomata, ecotype, gas exchange
Poster #267. NADPH-dependent thioredoxin reductase A (NTRA) confers elevated tolerance to oxidative stress and drought (Submission 361)
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NADPH-dependent thioredoxin reductases (NTRs) are key-regulatory enzymes determining the redox state of the thioredoxin (Trx) system that provides reducing power to peroxidases or oxidoreductases. Moreover, it also plays an essential function in the direct reduction of ROS and acquiring stress tolerance in plant. Cytoplasmic NTRA, mitochondrial NTRB, and chloroplastic NTRC are the three conserved NTRs which cooperate with specific subcellularly localized Trxs in Arabidopsis. However, cytosolic NTRs such as NTRA in Arabidopsis have not previously been identified in plants or mammals as a source of functional redundancy with mitochondrial NTRs. Here, we show the involvement of NTRA in the plant stress response counteracting oxidative and drought stresses. Methyl viologen (MV), an inducer of oxidative stress in plants, enhanced the NTRA transcripts. To identify the physiological role of NTRA influencing ROS homeostasis by stress, NTRA overexpression (NTRAOX) and knock-out mutants (ntra-ko) were generated. After exposure to oxidative stress, wild-type and ntra-ko plants were sensitive, but NTRAOX plants tolerant. ROS range was increased by MV in wild-type and ntra-ko plants, but not in NTRAOX. Investigating the involvement of Arabidopsis NTRA in drought, NTRAOX plants exhibited extreme drought tolerance with high survival rates, lower water loss and reduced ROS compared to wild-type and ntra-ko plants. Transcripts of drought-responsive genes, such as RD29A and DREB2A, were highly expressed under drought and antioxidant genes, namely CuZnSOD and APX1 were enhanced in the absence of drought in NTRAOX plants. The results suggest that NTRA overexpression confers oxidative and drought tolerance by regulation of ROS amounts.

Keywords: NADPH-dependent thioredoxin reductase A, Reactive oxygen species, Antioxidant, Oxidative stress, Drought stress, Water deficit

Poster #268. Collection of the response data to abiotic stress from Arabidopsis closely related species stored in RIKEN BRC (Submission 362)
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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 BioResource Project (NBRP) supported by the Japanese government (http://sassc.epd.brc.riken.jp/sassc/). To the classification of Arabidopsis closed species, we have tried to collect their DNA sequences of the chloroplast genome regions, rbcL and matK. These DNA sequences can be used for construct the phylogenetic tree and provide us the information on a classification of Arabidopsis closed species. We think Arabidopsis closed species are essential materials for the research on evolution and adaptation to the environment. We selected 19 species to collect the response data to abiotic stress treatment based on the classification data and seed availability. The sodium chloride treatment is our first target. We believe that the phenotype data will be useful for research communities. The data will be available from our database.

Keywords: bioresource, Arabidopsis closely related species, abiotic stress

Poster #269. Arabidopsis Cortical Microtubule- and Nucleus-localized RING E3 Ubiquitin Ligase AtAIRP4 is Positively Involved in ABA-mediated Germination Arrest and Stomatal Closure (Submission 364)
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As a sessile organism, plants have various defense mechanisms to survive under detrimental conditions. Diverse molecular and cellular processes are involved in the defense mechanisms, such as adjustments of transcriptome, proteome, nutrients and hormones, and rearrangement of cellular organelles. The ubiquitination and 26S proteasome system is one of the important processes to fine-tune proteome in higher plants. To study the relationships between ubiquitination and stress responses, we previously collected 100 different T-DNA insertion
mutants, in which RING-type E3 ubiquitin (Ub) ligase genes were suppressed, and screened the phenotypes of these mutants in terms of responses to abiotic stress and stress hormone ABA. One of the mutants, atairp4 (Arabidopsis thaliana ABA insensitive RING protein 4), showed a hyposensitive response toward ABA in seed germination. This mutant also showed partial impairment of ABA-mediated stomatal closure. AtAIRP4 has the C3H2C3-type RING domain at C-terminus of the protein and bacterially-expressed MBP-AtAIRP4 recombinant protein showed E3 Ub ligase activity in vitro. When the AtAIRP4-sGFP fusion construct was transiently expressed in Arabidopsis and tobacco protoplasts under the control of the 35S promoter, green fluorescence signal localized to a cytosolic microtubule and nucleus. The fluorescence signal of AtAIRP4-sGFP was also detected as a similar tubular pattern in guard cells of 35S:AtAIRP4-sGFP transgenic plants. Collectively, our data suggested that a cortical microtubule- and nucleus-localized AtAIRP4 RING E3 is involved in ABA-mediated germination arrest and stomatal closure as a positive regulator.

Keywords: ABA, RING E3 ubiquitin ligase, Cortical microtubule, seed germination, stomatal closure

**Poster #270. Functional Analysis of DREB1-type Transcription Factors under abiotic stress in Soybean** (Submission 367)
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Soybean (Glycine Max) is an important crop that is widely cultivated through the world, providing a valuable source of protein and oil for human consumption, animal feed, and biofuels. Soybean growth and yield are adversely affected by various environmental stresses such as cold, heat, drought, and high salinity. A cis-acting element DRE/CRT plays an important role in regulating gene expression in response to the stresses. Arabidopsis genes encoding DRE-binding proteins, DREB1/CFB and DREB2, function as transcriptional activators in the different stress-responsive gene expression, cold and drought, respectively. In this study, 14 DREB1-type transcription factors, GmDREB1s were identified from a soybean genome database. The expression of most GmDREB1 genes in soybean was extremely induced not only cold but also drought, high salt and heat stresses. The GmDREB1 proteins were activated transcription through DRE in Arabidopsis and soybean protoplasts.

Transcriptome analysis using transgenic Arabidopsis plants overexpressing the GmDREB1s indicates that their downstream genes overlapped with those of Arabidopsis DREB1A. We then analyzed the downstream genes of GmDREB1 using a transient expression system in soybean protoplasts. Many genes were up-regulated by the overexpression of GmDREB1, and DRE was the most conserved element in the promoters of these genes. However, among the downstream genes of GmDREB1, we found many soybean-specific stress-inducible genes, indicating that the GmDREB1 proteins regulate soybean-specific stress responsive genes under various abiotic stress conditions.

Keywords: Soybean, environmental stress, transcription factor

**Poster #271. The Arabidopsis RING E3 Ubiquitin Ligase AtAIRP2 Positively Regulates ABA-mediated Drought Response via the Interaction with the Possible Substrate Protein CAS1** (Submission 368)
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An environmental stress-induced protein turn-over performed by the ubiquitin (Ub)-26S proteasome system (UPS) is implicated in various abiotic stress adaptations in plants. The ubiquitination process is mediated by the cascade of E1, E2, and E3 Ub ligase. In general, E3 Ub ligases determine the specificity of substrate proteins. Here, we identified an E3 ligase, AtAIRP2 (for Arabidopsis ABA-Insensitive RING protein 2), and CAS1 (for a Candidate of AtAIRP2 Substrate Protein 1) as a possible substrate protein of AtAIRP2. AtAIRP2 encodes a cytosolic C3HC4-type RING E3 Ub ligase whose expression was induced by ABA and dehydration stress. The AtAIRP2 over-expressing transgenic (35S:AtAIRP2-sGFP) and atairp2 loss-of-function mutant plants exhibited hypersensitive and hyposensitive phenotypes, respectively, toward ABA in terms of seed germination, root growth, and stomatal movement. 35S:AtAIRP2-sGFP plants were highly tolerant to drought stress, and, in contrast, atairp2 mutant plants showed more susceptible phenotype than wild-type under dehydration stress. We identified CAS1, an
interacting protein of AtAIRP2, through yeast two-hybrid screening. The stability of ectopically expressed CAS1-myc recombinant protein was enhanced by MG132, a 26S-proteasome inhibitor, in N. Benthamiana leaves. Moreover, CAS1 knock-down transgenic plants showed more delayed growth at germination and seedling stages as compared to wild-type plants on ABA-supplemented medium. Overall, these results suggest that AtAIRP2, a C3HC4-type RING E3 Ub ligase, is a positive regulator in the Arabidopsis ABA-dependent drought response and that CAS1 could be a substrate of AtAIRP2.

Keywords: AtAIRP2, E3 ligase, ubiquitination, drought stress, ABA-dependent drought response, ubiquitin-26S proteasome system

Poster #272. Molecular Mechanisms of Temperature-Dependent Crossovers in Arabidopsis thaliana (Submission 371)
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Meiotic recombination establishes physical connections between chromosomes and results in a reciprocal exchange of genetic material (crossovers) between homologous chromosomes. In Arabidopsis, crossovers originate from either the Type I or Type II pathway; Type I crossovers comprise the majority of crossovers in Arabidopsis and are sensitive to the placement of adjacent crossovers whereas Type II crossovers are not. Crossover frequency is thought to be subject to homeostatic regulation, however studies have suggested that the frequency of recombination can be modulated by a variety of factors, such as environmental conditions. Specifically, an increase in temperature has been shown to increase recombination frequency in Arabidopsis. In this study, we utilize a pollen tetrad assay to measure crossover frequency in Arabidopsis msh4 and mus81 mutants that separately disrupt the function of the Type I and Type II pathways respectively. Preliminary results suggest that these crossovers originate from the Type I pathway. These results provide novel insight into the molecular mechanics of environmentally-induced changes in recombination frequency.

Keywords: meiotic recombination, crossovers, temperature, interference

Poster #273. Functional analysis of the Hikeshi-like protein that interacts with HSP70 in Arabidopsis (Submission 381)
Shinya Koizumi¹, Naohiko Ohama¹, Junya Mizoi¹, Kazuo Shinozaki², Kazuko Yamaguchi-Shinozaki¹
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As sessile organisms, plants are exposed to various environmental stresses, among which heat is one of the most critical stressors for the growth and productivity of plants. Heat stress damages plant cells by provoking protein aggregation, reactive oxygen species (ROS) production, and the hyperfluidization of membranes. To adapt to elevated temperatures, plants have developed a wide range of strategies at the cellular and molecular levels. The expression of heat shock proteins (HSPs) is the most highly conserved mechanism for protecting cells from heat shock damage. HSPs interact with damaged proteins and assist in properly refolding them. The 70 kDa heat shock proteins, HSP70s are central components of the cellular network of molecular chaperones. Five cytosolic/nuclear HSP70s have been identified in Arabidopsis, and their amino acid sequences are highly conserved. Because cytosolic/nuclear HSP70 isoforms most likely function in a redundant manner, loss-of-function analyses of cytosolic/nuclear HSP70 may be difficult. Consequently, little is known about HSP70-mediated cellular stress responses in plants despite their essential role in thermotolerance. Recently, an HSP70 nuclear import carrier protein named Hikeshi was identified in human cells. Hikeshi plays an important role in cellular protection against heat-induced damage via the nuclear translocation of HSP70 in human cells. Because this Hikeshi-mediated HSP70 nuclear import pathway appeared to be a fundamental mechanism for heat stress tolerance, we searched for a Hikeshi ortholog in the Arabidopsis genome. In this study, we identify an Arabidopsis ortholog to Hikeshi, Hikeshi-like protein (HKL), and show that it interacts with Arabidopsis HSP70 isoforms and is involved in the regulation of the subcellular localization of HSP70. Our histochemical expression analysis reveals that HKL is predominantly expressed in meristematic tissues in a heat-dependent manner. Moreover, transgenic plants overexpressing HKL showed higher thermotolerance. Our results suggest that HKL plays a positive role in the protection of cells under heat stress in cooperation with HSP70.
proteins.

Keywords: heat stress, heat shock protein, thermotolerance, nuclear carrier protein

**Poster #274. Identification of cis-acting elements in dehydration-inducible promoters in Zea mays and Glycine max** (Submission 382)

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The 2,400-Mb maize (Zea mays) genome and the 1,115-Mb soybean (Glycine max) genome have been sequenced, and 39,389 and 46,430 protein-coding genes, respectively, have been predicted. In Arabidopsis, cis-acting promoter elements involved in dehydration-responsive gene expression have been extensively analyzed; however, the characteristics of such cis-acting promoter sequences in dehydration-inducible genes of maize and soybean remain to be clarified. In this study, we performed CAGE (cap analysis of gene expression) and microarray analyses using maize and soybean, and compared characteristics of identified dehydration-inducible genes and promoters. Transcription profiles of the dehydration-responsive genes were similar in maize and soybean, showing representative up-regulated (dehydrin/LEA) and down-regulated (photosynthesis-related) genes. All (46 = 4096) hexamer sequences in the promoters of maize and soybean were investigated, revealing the frequency of conserved sequences in dehydration-inducible promoters. A core sequence of the abscisic acid-responsive element (ABRE) was the most conserved in dehydration-inducible promoters of maize and soybean, suggesting that transcriptional regulation for dehydration-inducible genes is similar in maize and soybean, with the ABRE-dependent transcriptional pathway. In addition, we identified novel highly conserved sequences in dehydration-inducible maize promoters that were not highly conserved in dehydration-inducible promoters in Arabidopsis and soybean. These sequences have never been reported as cis-acting elements for dehydration-inducible gene expression, and thus they are novel candidates for cis-acting elements involved in dehydration-inducible gene expression in maize.

Keywords: crop, Zea mays, Glycine max, CAGE, promoter, cis-acting elements, dehydration

**Poster #275. Transcriptional regulation of oxidative stress related genes plays an important role for cadmium tolerance in Arabidopsis thaliana** (Submission 385)

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Cadmium (Cd) is a heavy metal pollutant widespread in industrial and agricultural areas. It is toxic to plants, fungi, animals and humans. Some transporters and thiol-containing chelators have been reported to contribute to Cd tolerance of plants, but there is scanty knowledge on transcriptional regulation of cadmium tolerance. To identify the transcription factor for cadmium tolerance, we screened 1600 of A. thaliana CRES-T (Chimeric REpressor gene Silencing Technology) lines and found several CRES-T lines that displayed more tolerant phenotype in cadmium containing media than wild type. One of the lines appeared several times during screening. We identified the transcription factor in this CRES-T line and named it as cadmium tolerant factor (CTF). CTF knockdown plants exhibited tolerance to high cadmium, and decreased reactive oxygen species level in the root detected by DCF staining, whereas CTF overexpressing plants exhibited the opposite. The expression of CTF was induced within 2 hours after cadmium treatment at both RNA as well as protein level. Translation fusion of GUS reporter showed that CTF is specifically expressed at root and shoot apical meristems, young flowering buds and senescing leaves. When expressed under own promoter and fused to GFP was localized in the dots in nucleus. Analysis with luciferase reporter gene revealed that CTF has a transcriptional activation activity. The downstream target genes of CTF were identified based on microarray analysis, yeast 1 hybrid and luciferase assay. The targets included a member of oxysterol binding protein family and AP2 transcription factor, which have been annotated to be involved in oxidation-reduction process. Moreover, homozygous knockout of each target exhibited slight tolerance to Cd, supporting the possibility that these genes are indeed target genes of CTF, contributing negatively to cadmium tolerance. Taken together, our data indicate that Arabidopsis CTF
regulates Cd tolerance level by modulating genes that are important for ROS scavenging processes.

Keywords: Cadmium, Transcription Factor (CTF), Oxidative stress, React Oxygen Species (ROS)

**Poster #276. The photoperiod pathway modulates flowering time in response to nitrate.** (Submission 435)
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Nitrogen (N) is an important macronutrient required for plant growth and development. N nutrient/metabolites can have a profound impact on root development, flowering time and other developmental programs. The flowering is the most critical aspect to ensure reproductive success. Plants have evolved complex regulatory mechanisms to bloom when conditions are most favorable. Although the mechanism that controls the transition to flowering has been widely investigated, the underlying regulatory factors controlling flowering time in response to nitrate remain incompletely understood. In this study, we used systems biology and molecular genetics approaches to identify regulatory factors involved in N-control of flowering time in Arabidopsis. Our systems analyses suggest N signals interact with photoperiod pathway to control flowering time in Arabidopsis. We showed that these genes are regulated by nitrate. Genetic analyses of loss-of-function mutants indicate that these genes are important regulators of flowering time in response to nitrate. Our work defined a model for nitrate control of flowering time in Arabidopsis thaliana.

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Keywords: Flowering time, Nitrate, Gene expression

**Poster #277. Differential roles of the Arabidopsis Synaptotagmins 1 and 3 in Cold Acclimation and Freezing Tolerance** (Submission 467)
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In Arabidopsis, the phospholipid binding protein synaptotagmin1 (SYT1) participates in the Ca²⁺-dependent freezing tolerance acquisition. In this report we demonstrate the housekeeping role of SYT1 in both cold acclimation and freezing tolerance under physiological conditions, and evaluate the potential role of additional members of the SYT family in those processes. As a result, we identify the previously reported synaptotagmin 3 (SYT3) pseudogene as a cold inducible determinant essential for cold acclimation on SYT1 deprived backgrounds. As SYT1, SYT3 is a Ca²⁺-dependent phospholipid binding protein, but in contrast to the high and ubiquitous SYT1 expression, SYT3 expression is low and mainly restricted to meristemoids, young stomata, and old primary root. Both differential expression levels and tissue specificity suggest that SYT1 and SYT3 might have functional specialization in response to cold acclimation and freezing stress. Finally, our study highlights the importance of these phospholipid binding proteins as regulatory components in specific cold signaling pathways.

Keywords: Plant synaptotagmins, Cold acclimation, Freezing tolerance

**Poster #278. Responses of Arabidopsis peroxidase RNAi lines to oxidative stresses** (Submission 482)
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Peroxidases are accountable for a variety of plants' physiological and developmental processes including defense mechanism, lignification, suberization, auxin catabolism and wound healing. Peroxidases are categorized in accordance with their substrate specificity and origin. Class III peroxidases are plant-specific oxidoreductases, belonging to a superfamily that contains three different subclasses of peroxidases. Arabidopsis genome harbors
73 members of class III peroxidases (EC 1.11.1.7). Of these 73 genes, here we report four genes that are co-regulated by both light and brassinosteroid (BR) signaling pathways. We observed that our peroxidase RNAi lines, which have been constructed using RNAi cassettes, specifically affect lignin content and composition. Lignification is important in plants for resistant to biotic and abiotic stresses. Thus, we examined the changes in phenotypes of wild type and RNAi lines upon oxidative stresses induced by drought, salt, heat and low temperature. To elucidate the correlation between peroxidase's enzymatic activities and plants' anti-oxidative responses, we measured the amount of hydrogen peroxide (H2O2) and corresponding activities of peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) in seedlings of wild type and RNAi lines upon the abiotic stresses. Furthermore, immunohistochemical staining methods were used to demonstrate time series changes in tissue development in response to the stresses. The results will contribute to thorough understanding of plant antioxidant enzymes and their responses to the environment.

Keywords: peroxidase, RNAi, oxidative stress

Epigenetics/Chromatin

Poster/Talk #279. Epi-genomic analyses of stem cells in Arabidopsis thaliana (Submission 84)
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Stem cells are pluripotent cells that are able to self-renew and differentiate into various tissues. Plants harbor distinct stem cell niches that generate the different organs throughout the life cycle. Epigenetic gene regulation is an important mechanism for the maintenance of cell fate. Studies in human and mouse cells have indicated that stem cells regulate their pluripotent status by subjecting repressive histone modifications on important regulatory genes. In plants, loss of function of Polycomb group (Pc-G) proteins that catalyse the repressive mark histone 3 lysine 27 tri-methylation (H3K27me3) cause strong defects in cell identity.

To study the role of chromatin-based gene regulation in maintaining stem cell identity, we have isolated stem cells of the shoot apical meristem with the INTACT method¹ and performed chromatin immunoprecipitation (ChIP) using antibodies against histone modifications. By conducting ChIP-sequencing, we found that hundreds of genes are differentially targeted by H3K27me3 in stem cells and differentiated cells. Among those genes, the genes involved in organ development and regulations of metabolic process are significantly enriched. Interestingly, ChIP-qPCR analyses of both stem and differentiated cells revealed that the stem cell specific gene CLAVATA3 (CLV3) is targeted by both the repressive mark H3K27me3 and the active mark H3K4me3 in stem cells, while CLV3 is only targeted by the repressive mark H3K27me3 in differentiated cells, suggesting that CLV3 could be bivalently marked in stem cells.

Reference

Keywords: ChIP-seq, INTACT, Stem cell, H3K27me3, H3K4me3, Epi-genome

Poster #280. Two RNA binding proteins are integral part of the HUB1/2 complex (Submission 105)
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HISTONE MONOUBIQUITINATION1 (HUB1) is an unconventional ubiquitin E3 ligase involved in histone H2B modification implicated in transcription activation in plants during periodic and inducible processes such as cell cycle, dormancy, flowering time and defense responses. HUB1 interacting proteins were investigated by means of Tandem Affinity Purification and Yeast-2-Hybrid. In addition to confirming the dimerization of HUB1 and HUB2, we newly identified two proteins as integral part of the HUB1/2 complex. The two interactors of the HUB1/2 complex contain putative protein domains involved in RNA binding. Localization of HUB1, HUB2 and interactors was
established by transient GFP fluorescence in tobacco leaves and by confocal microscopy of primary roots of stable transgenic Arabidopsis lines. The interactors were exclusively located in the nucleus, while HUB1 and HUB2 were shown to localize in the nucleus and the cytoplasm. Multi-probe in situ hybridization visualized a strong co-expression of HUB1 and two interactors in the shoot apical meristem, one of the interactors and HUB1 are co-expressed in expanding leaves while the other one and HUB1 are co-expressed in leaf primordia and vascular tissue. Interactors were functionally characterized and compared with HUB1/2 by analysis of vegetative growth and flowering time of respective mutants. We found that the four proteins play common roles in vegetative growth control while flowering time was oppositely affected in hub1/2 and one interactor mutant suggesting antagonistic roles of HUB1/2 and interactor in flowering regulation.

Keywords: histone monoubiquitination, HUB1, RNA binding proteins, flowering regulation

Poster/Talk #281. NTR1 is involved in splicing check-points formation and RNA Pol II pausing at alternative splicing sites in Arabidopsis (Submission 106)
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Splicing is a highly complicated process that involves more than 200 proteins and five small RNAs associated with the spliceosome at different stages of splicing. This enormous number of proteins is reflected in the number of molecular processes, including transcription, that are intertwined with splicing. Several models have been proposed to explain how chromatin regulates alternative splicing, including the direct sensing of histone markers by spliceosome-associated factors and the effect of the transcription elongation rate on alternative splice site selection. We have identified an Arabidopsis homologue of NTR1 (AtNTR1), a conserved spliceosomal disassembly factor, as a protein required for co-transcriptional pausing at alternative splice sites. In our studies we analyzed 144 alternative splice events in atntr1 and we found that the most abundant splicing defects were intron retention and exon skipping. We observed also strong selection towards distal splice sites. Proximal/distal splice site selection has been proposed to depend on the RNA Polymerase II elongation rate. Therefore, the bias we observed could indicate a defect in transcription elongation in the atntr1 mutant. To test the relationship between AtNTR1 and PolII, we analyzed ATNTR1 immunolocalization. We confirmed previous results from human cells showing that NTR1 is localized in the nucleus but excluded from the nucleolus. Using dual labeling we shown strong co-localization of ATNTR1 with Ser2-phosphorylated PolII which is to mark actively transcribing PolII associated with gene bodies. In agreement with the altered elongation rate in atntr1, we demonstrate that the majority of splicing defects caused by the lack of ATNTR1 are opposite to changes observed in the TFIIS mutant, in which endonucleolytic cleavage by PolII is blocked. In addition, atntr1 shows decreased PolII occupancy at the majority of the alternatively misspliced exons and introns.

Splice sites are sites of transient PolII pausing induced by splicing itself rather than recruitment of the spliceosome. This pausing has been proposed to constitute a quality checkpoint for splicing, which provides feedback on transcription. Our data are consistent with this suggestion and show that ATNTR1 is required for localized transcriptional pausing at the affected alternatively spliced exons and introns and play important role in quality proofreading of splicing.

Keywords: alternative splicing, co-transcriptional splicing, splicing proofreading, RNA Polymerase II

Poster #282. Functional characterization of BLISTER - A Polycomb group associated protein in Arabidopsis (Submission 108)
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Epigenetic gene silencing in Arabidopsis thaliana is mediated by at least three different PRC2 (POLYCOMB
REPRESSIVE COMPLEX 2) complexes, which repress their target genes by tri-methylating (me3) histone H3 at lysine (K) residue 27 (H3K27me3). The plant specific protein BLISTER (BLI) interacts with the PRC2 methyltransferase component CURLY LEAF (CLF) (Schatlowski et al., 2010). Loss of BLI results in a pleiotropic phenotype with defects in seed, leaf and flower development, and a reduced transmission through both germlines. BLI prevents premature differentiation but also shows outgrowing cells ("blisters") on several organs, indicating a loss of cell identity. In the bli mutant Polycomb group (PcG)-target genes are significantly overrepresented in the set of misexpressed genes, suggesting that BLI has an important function in PcG-mediated gene silencing. Surprisingly the bli mutant does not show reduced H3K27me3 levels at several PRC2-target genes tested. This suggests a second level of PcG-mediated gene silencing, in which BLI could act downstream of PRC2 to stably silence genes carrying H3K27me3. BLI does not only localize to the nucleus (like PRC2 proteins) but also co-localizes with proteins marking the endoplasmic reticulum (ER) and processing-bodies in Arabidopsis root cells and tobacco epidermal cells. The localization of BLI at the ER coincides with upregulation of ER-stress responsive genes in the bli mutant. Also genes involved in responses to the plant hormone abscisic acid (ABA) and cold-responsive genes are misregulated in bli mutants, indicating that BLI might act as a general stress response regulator. To determine how different domains of BLI affect its sub-cellular localization and function, these were mutated and transgenic lines were generated to analyze the effect of the mutation on BLI localization and complementation of the phenotype. In summary, I will present approaches to elucidate (i) the role of BLI in PcG-mediated gene silencing, (ii) its role in stress response regulation and (iii) to analyze the function of BLI protein domains on both sub-cellular localization and complementation of the phenotype. We hypothesize that BLI is a regulator of a specific, possibly stress-responsive set of PcG target genes but also has functions independent of PcG.

Keywords: Polycomb, H3K27me3, stress, epigenetic

Poster #283. Epigenetic regulator AS1-AS2 is involved in gene body DNA methylation of ETT in establishment of leaf adaxial-abaxial polarity in Arabidopsis thaliana (Submission 143)
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Leaves develop as lateral organs from the peripheral zone of a shoot apical meristem. Initially, a group of cells is patterned along the proximal-distal axis and then establishment of the adaxial-abaxial axis is crucial for further leaf development. Subsequent cell proliferation along the medial-lateral axis results in flat and mediolateral symmetric leaves. The ASYMMETRIC LEAVES1 (AS1)-AS2 nuclear-protein complex repress class 1 KNOX genes to pattern along the proximal-distal axis. In addition, The AS1-AS2 nuclear-protein complex has the crucial role in leaf adaxial-abaxial polarity specification. The AS1-AS2 complex directly binds the promoter region of AUXIN RESPONSE FACTOR3/ETTIN (ARF3/ETT) gene, which promotes abaxial identity, and represses the expression of ETT (Iwasaki et al., 2013). The AS1-AS2 complex also indirectly represses both ETT and ARF4 by post-transcriptional gene silencing (PTGS) via the miR390-RDR6-tasiRNA pathway. However, information on mechanisms controlling AS1-AS2 downstream gene ETT has remained elusive. Although it has been reported that the AS1-AS2 complex maintains repression of KNOX homeobox genes via direct recruitment of Polycomb-repressive complex2 (Lodha et al., 2013), mechanisms involved in epigenetic regulation during leaf adaxial-abaxial polarity specification are still unknown. We found that AS1-AS2 maintains the status of DNA methylation in the ETT coding region, but not in ARF4. In agreement, filamentous leaves formed in as1 and as2 treated with DNA methylation inhibitors 5-aza-2'-deoxycytidine (5-aza-2'dC) and zebularine were rescued by loss of ETT. In addition, as1 and as2 mutants treated with DNA methylation inhibitors 5-aza-2'dC and zebularine was decreased DNA methylation frequency of the ETT coding region. We will discuss roles of the ETT gene body DNA methylation on leaf development in A. thaliana.

Keywords: Gene body methylation, 5-aza-2'-deoxycytidine (5-aza-2'dC), Leaf development, AUXIN RESPONSE FACTOR3/ETTIN (ARF3/ETT), ASYMMETRIC LEAVES1 (AS1), ASYMMETRIC LEAVES2 (AS2)
Poster/Talk #284. Arabidopsis MSH1 mutation alters the epigenome and produces heritable changes in plant growth (Submission 170)
John Laurie¹, Ying-Zhi Xu¹, Kamaldeep Virdi¹, Dong Wang¹, Yashitola Wamboldt¹, Mei Chen¹, Jean-Jack Riethooven¹, Jian Tao Yu¹, Suhua Feng², Mon-Ray Shao¹, Robersy Sanchez¹, Maria Arrieta-Montiel¹, Hardik Kundariya¹, Vikas Shedge³, Sally Mackenzie¹
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Plants respond to environmental stresses using various means, including epigenetic modulation. Previously we reported that mutation of the nuclear-encoded MutS Homolog 1 (MSH1) gene causes heritable changes in plant growth habit, including dwarfing, delayed flowering and leaf variegation. The MSH1 protein is targeted to both mitochondria and plastids and is known to suppress mitochondrial genome recombination. MSH1 expression is highest in reproductive tissues and decreases sharply upon abiotic stress. Here we show that crossing Arabidopsis msh1 mutant plants to isogenic wild type results in enhanced growth progeny and that the enhanced growth vigor is heritable. DNA methylation profiling reveal a tendency for mutants to become hypermethylated in both the CG and CHG contexts, with greater CHG hypermethylation appearing in dwarfed plants. The bulk of the CHG changes fall on sequences matching transposable elements, with a preference for Gypsy-like retroelements. Following crossing and generation of enhanced growth lines, however, transposable elements tend to become more hypomethylated than hypermethylated in CG and this change is accompanied by a concomitant increase in CHH methylation. A striking feature of these lines is that the hypermethylation of CHG that first appeared in dwarfed mutants is retained following crossing. Collectively, our results point to epigenetic perturbations in msh1 mutants that are both retained and reprogrammed in resulting enhanced growth progeny after crossing. Transposable elements appear to play an active role in this phenomenon showing signatures of activation followed by resilencing in enhanced growth progeny. A role for MSH1 in environmental sensing and transgenerational epigenetic memory will be discussed.

Keywords: MSH1, DNA methylation, Plastid, Epigenetics, Transposable elements

Poster/Talk #285. NATURAL VARIATION IN ARABIDOPSIS ATFH5 EXPRESSION INDICATES AN ADAPTIVE ROLE FOR POLYCOMB REGULATION OF THE SEED ENDOSPERM. (Submission 181)
Jonathan Fitz Gerald¹, Ellen Dahl¹, Brandon Smith¹
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Polycomb group (PcG) complexes have been associated with cellular memory of positional identity during early development. The seed endosperm of Arabidopsis thaliana, provides a simple model for understanding this role of PcG. In Landsberg erecta (Ler) and Columbia (Col) ascensions, the maternally expressed imprinted reporter gene AtFH5::H2B-RFP is expressed solely in the posterior chalazal cyst of the endosperm. Silencing of the reporter in the anterior and peripheral endosperm requires PcG function. Interestingly, when Ler and Col are crossed, segregants carrying the AtFH5 reporter exhibit a range of phenotypes from no RFP expression to expression throughout the endosperm similar to PcG loss of function. Collectively, our results point to epigenetic perturbations in msh1 mutants that are both retained and reprogrammed in resulting enhanced growth progeny after crossing. Transposable elements appear to play an active role in this phenomenon showing signatures of activation followed by resilencing in enhanced growth progeny. A role for MSH1 in environmental sensing and transgenerational epigenetic memory will be discussed.

Keywords: parental genomic imprinting, Polycomb, formin, seed endosperm, natural variation

Poster #286. Sequence-specific nucleosome positioning in putative transcription factor binding sites in Arabidopsis thaliana (Submission 189)
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Nucleosomes positioning has been shown to influence the access of transcription factors (TFs) to their binding sites and thus to regulate gene expression in mammalian and fungal models. To advance our limited knowledge on the relationship between nucleosome occupancy and potential TF binding sites in plants, we mapped nucleosome positions in the Arabidopsis thaliana genome and found that nucleosome occupancy was negatively correlated with gene expression. To identify sequence motifs located in nucleosome-depleted regions that are potential TF binding sites, the nucleosome occupancy on 6-mer motifs was examined. Among 4,096 6-mers, 655 have significantly lower nucleosome occupancy than the background level. These nucleosome-depleted motifs tend to have significantly higher GC content and are tightly associated with DNase I hypersensitivity sites, consistent with the characteristics of mammalian TF binding sites. In addition, the depletion tends to be in motif sites located 50-150bp upstream of transcriptional start sites but not in coding regions, introns, or untranslated regions. The nucleosome-depleted motifs include known cis-elements, e.g. the G-box motif (CACGTG) recognized by basic helix-loop-helix transcription factors. In contrast, a motif with one nucleotide substitution (AACGTG) is no longer depleted of nucleosomes, suggesting that nucleosome depletion is highly sequence-specific. Taken together, our study provides the first comprehensive study accessing nucleosome occupancy on sequence motifs and its association with gene expression in plants. The integration of nucleosome occupancy and sequence motif information allows us to establish a comprehensive cis-regulatory model for expression prediction that will also be discussed.

Keywords: nucleosome, motif discovery, transcription factor binding site, gene expression

Talk #287. Histone Modifications and Histone Code in Brassinosteroid Regulated Gene Expression (Submission 212)
Yanhai Yin\(^1\), Xiaolei Wang\(^2\), Jiani Chen\(^1\), Zhouli Xie\(^1\), Sazhen Liu\(^1\), Trevor Nolan\(^1\), Patrick Schnable\(^1\), Zhaohu Li\(^2\), Hongqing Guo\(^1\)

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Histones can be modified at multiple positions, which forms "histone codes" that determine gene activity. How histone modifications and histone codes mediate hormonal responses is not well understood in plant systems. The plant steroid hormone, brassinosteroid (BR), plays important roles in plant growth, development and responses to environmental stresses. BR signals through receptors localized to the plasma membrane and other signaling components to regulate the BES1/BZR1 family of transcription factors, which modulates the expression of thousands of target genes. We have previously found that Histone 3 lysine 27 (H3K27) demethylase REF6 is involved in BR-induced gene expression (PNAS, 2008 105: 7618). We have recently found that histone lysine methyltransferase SDG8, implicated in H3K36 di- and tri-methylation (H3K36me2 and me3), is involved in BR-regulated gene expression. BES1 interacts with SDG8, directly or through IWS1, a transcription elongation factor involved in BR-regulated gene expression (PNAS, 2010 107: 3918). The knockout mutant of SDG8 gene, sdg8, displays a reduced growth phenotype with compromised BR responses. Global gene expression studies demonstrated that while BR regulates about 5000 genes in wild-type plants, the hormone regulates less than 700 genes in sdg8 mutant, demonstrating that SDG8 plays a critical role in BR-regulated gene expression. In addition, more than half of BR-regulated genes are differentially affected in sdg8 mutant. A Chromatin Immunoprecipitation (ChiP) experiment showed that H3K36me3 is reduced in BR-regulated genes in the sdg8 mutant. Based on these results, we propose that combination of histone modifications including H3K27 and H3K36 methylation form a histone code to dictate BR-regulated gene expression. The research is supported by National Science Foundation of USA (IOS 1257631) and National Science Foundation of China (No. 30825028).

Keywords: Histone modifications, Histone code, Hormone signaling, Brassinosteroid, Histone Methyltransferase SDG8, Histone demethylase REF6, Gene expression, Chromatin

Poster/Talk #288. Silencing Initiation: Recognizing an Exogenous Transposon (Submission 233)
Dalen Fultz\(^1\), Keith Slotkin\(^1\)

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Transposable elements (TEs) are mobile, parasitic DNA elements found throughout all life. When active, they cause mutations and destabilize chromosomes. These alterations can damage the germline and cause or
accelerate disease, and in many eukaryotes, TEs and remnant sequences make up large portions of the genome. Eukaryotes have developed several homologous small RNA (sRNA) and epigenetic systems to control the expression and transposition of TEs. However, little is known about initial TE recognition by the host. How does an organism differentiate self from non-self sequence in the nuclear genome? This is relevant for the silencing of TEs, transgenes, and viruses. Our lab has found that newly introduced copies of endogenous TEs are recognized by homology to existing copies, mediated by sRNAs. Other labs have transformed active Nicotiana tabacum retrotransposons into Arabidopsis thaliana. Despite a lack of homology to the host genome, these elements were not only recognized, but heritably silenced after introduction and transposition. However, there is little evidence for how this silencing was triggered or what pathways may be involved.

Using Agrobacterium-mediated transformation, we have introduced one of these TEs, Tto1, into Arabidopsis. Analysis of DNA methylation and sRNA has shown that Tto1 is quickly and deeply silenced in this first generation. This is dependent on the RNA-directed DNA Methylation pathway. Transcriptional induction of the element activates the RNA interference pathway (RNAi) against it. From sRNA northern blot, we infer that this TE is being recognized through its homology to endogenous TEs, in spite of its limited sequence similarity to any region of the Arabidopsis genome. However, when homology based recognition is eliminated, RNAi is still active against the expressed element. Using truncated forms of this TE, we have also seen that full silencing requires the entire element. In order to minimize homology, further studies will examine the recognition of yeast TEs.

Keywords: epigenetics, transposon, transposable element, siRNA, RNAi, RNA directed DNA Methylation

Poster/Talk #289. The EARLY SENESCENCE AND DWARF 1 (ESD1) Gene, Encodes an DNA Glycosylase, Functions as A Novel DNA Demethylase in Arabidopsis (Submission 234)
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By broad literature survey, we have developed a leaf senescence database (LSD, http://www.eplantsenescence.org/) that contains a total of 5356 senescence associated genes (SAGs) from 44 species. However, the regulatory role of most of the SAGs in leaf senescence is not known. By large scale screening of the altered senescence phenotype mutants deposited in the LSD database, we isolated a early senescence mutant (esd1), which also is dwarf compared to wild-type (WT). The ESD1 gene encodes an DNA glycosylase, and GO analysis indicates that ESD1 is involved in base-excision repair (BER). To investigate the molecular mechanisms of ESD1 in regulating plant development, RNA-sequencing analysis was performed and revealed that mutation in ESD1 globally affects gene expression pattern. Previous studies demonstrate that several DNA glycosylases are found to regulate gene expression by active demethylation in Arabidopsis. To determine whether ESD1 regulates gene expression via active demethylation pathway, whole genome bisulfite sequencing (WGBS) analysis was performed. However, we did not observe global DNA methylation changes in esd1 mutant plants: the overall methylation levels (CG, CHG and CHH) of WT and the esd1 mutant were very similar, indicating that ESD1 removes methylcytosines within special loci. Interestingly, we find that methylation levels in the promoter of CPS, a key player in gibberellic acid (GA) biosynthesis pathway, were higher in esd1 mutant than that in WT, suggesting that mutation in ESD1 leads to locus-specific DNA hypermethylation. Furthermore, the bioactive GA levels in esd1 are significantly lower than that in WT. Genetic analysis further reveals that loss-of-function of five DELLAs rescues the dwarf phenotype of esd1. Taken together, our data suggest that ESD1 functions as a novel demethylase and mutation in ESD1 decreases the contents of GA, a negative regulator of leaf senescence and a promoter of plant growth, and results in the early senescence and dwarf phenotype.

Keywords: Leaf Senescence, DNA Glycosylase, DNA Demethylation, Gibberellic Acid, Arabidopsis thaliana

Poster/Talk #290. Redox regulation of histone deacetylases (Submission 253)
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In response to stress but also during development plants synthesize small, redox-active molecules like nitric oxide or hydrogen peroxide which post-translationally modify target proteins thereby altering their catalytic activity,
subcellular localization or association with binding partners. Nitric oxide is emerging as one of the most important redox-signaling molecules in plants. It exerts most of its biological functions by binding covalently to cysteine residues of susceptible proteins resulting in the formation of S-nitrosothiols.

In humans it is well documented that some histone deacetylases - one of the oldest known enzyme families - are regulated by S-nitrosoylation and other redox modifications. HDACs are very important transcriptional regulators involved in a variety of physiological processes. They catalyze the removal of acetyl groups from acetylated lysine residues on histone proteins which leads to a compaction of chromatin and transcriptional repression of the corresponding genes. In plants, however, there is no evidence that HDACs might be regulated by nitric oxide or other redox molecules. We were interested in whether histone deacetylases are redox-regulated during pathogen response in Arabidopsis. We therefore incubated protoplasts generated from A. thaliana cell culture with salicylic acid to mimic a pathogen attack. SA treatment resulted in a strong and fast production of nitric oxide as previously described. Moreover, a significant decrease in total HDAC activity was observed, which was dependent on nitric oxide. Next it was examined whether HDAC activity in protoplasts and purified nuclear extracts is affected by S-nitrosoylation promoting chemicals like GSNO and SNAP. Both compounds inhibited HDAC activity in a concentration dependent manner, demonstrating that HDACs or proteins which regulate them are sensitive to S-nitrosoylation. Future work will concentrate on identifying the NO-regulated HDAC(s) and deciphering the physiological role of this modification.

Keywords: redox-regulation, histone deacetylases, S-nitrosoylation

**Poster #291. Chromatin remodelling in response to seasonal environmental signals during seed dormancy cycling** (Submission 283)
Steven Fotitt¹, Kerstin Mueller², Allison Kermode², William Finch-Savage¹
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Posttranslational modification of specific histone residues is a major epigenetic mechanism which, depending on which residues are modified, is associated with active transcription or transcriptional repression. We exploited the genetic diversity between the winter and summer annual Arabidopsis ecotypes Cvi and Bur to investigate the expression of genes involved in chromatin remodelling via histone ubiquitination/de-ubiquitination, histone acetylation/deacetylation, and histone methylation/demethylation over an annual temperature-cued dormancy cycle in the soil seed bank. We also investigated histone modifications on the major dormancy regulator DOG1 (DELAY OF GERMINATION1) in response to changing environmental conditions as seeds lose and re-enter dormancy under laboratory conditions. Seed dormancy cycling plays a crucial role in the timing of the plant life cycle of many species and requires the sensing and temporal integration of environmental signals such as temperature and light. Little is known about its regulation and the involvement of chromatin remodelling following seed dispersal into the soil. We found that ROS1 (REPRESSOR OF SILENCING1) expression is correlated negatively with soil temperature and positively with dormancy while KYP/SUVH4 (KRYPTONITE) expression was correlated positively with temperature and germination potential. We propose ROS1 dependent repression of silencing and KYP/SUVH4 dependent gene silencing is sequentially required for dormancy maintenance and suppression during dormancy cycling in the soil. Seasonal expression patterns of H2B modifying genes are correlated negatively with temperature and positively with DOG1 expression. Histone acetyltransferase genes are similarly correlated, with histone deacetylases positively correlated with temperature. During laboratory induced dormancy cycling H3K4me3 activating marks remained relatively stable along the DOG1 gene, but H3K27me3 repressive marks accumulated during the low dormancy phase of the dormancy cycle. We propose that accumulation of these histone modifications on DOG1 serves as a thermal memory during the annual dormancy cycle, and that chromatin remodelling plays a crucial role in temporal sensing of environmental conditions in seeds.

Keywords: chromatin remodelling, seed dormancy cycling, soil seed bank, integration of environmental signals

**Poster/Talk #292. ARGONAUTE10 promotes the degradation of miR165/6 through the SDN1 and SDN2 exonucleases in Arabidopsis** (Submission 292)
Lijuan Ji¹, Yu Yu², Jixian Zhai³, Elizabeth Luscher², Lei Gao², Chunyan Liu³, Xiaofeng Cao³, Beixin Mo⁴, Blake C.
Both biogenesis and degradation contribute to the steady-state levels of microRNAs (miRNAs). miRNA degradation in both plants and animals is associated with 3' truncation and 3' uridylation, and in Arabidopsis, the nucleotidyl transferase HEN1 SUPPRESSOR1 is responsible for 3' uridylation. The SMALL RNA DEGRADING NUCLEASE (SDN) family of 3' to 5' exonucleases degrades short RNAs in vitro and limits the accumulation of miRNAs in vivo, but it is not known whether SDNs are responsible for the accumulation of 3' truncated miRNA species in vivo. In addition, there has not been an example of active miRNA turnover being critical to specific developmental processes. Arabidopsis ARGONAUTE10 (AGO10) maintains stem cell homeostasis in meristems by repression of miR165/6, a conserved miRNA acting through AGO1. Here, we report that AGO10, through binding to miR165/6, reduces miR165/6 accumulation by enhancing its degradation. We show that SDNs are responsible for the generation of 3' truncated miRNAs in vivo and AGO10 promotes the 3' truncation of miR165/6 by SDNs. Our work uncovers a novel mechanism of miRNA degradation mediated by an argonaute protein, which contrasts the known stabilizing effects of most AGO proteins on their associated miRNAs. This work, together with previous studies on AGO10, demonstrates that spatially regulated miRNA degradation underlies stem cell maintenance in plants.

Keywords: AGO10, degradation of miR165/6, SDN, Arabidopsis

Poster/Talk #293. A chromatin switch underlies flower primordium initiation downstream of AUXIN RESPONSE FACTOR 5/MONOPTEROS (Submission 377)
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Reprogramming of cell identities during development is enabled by changes in the accessible genome in the context of chromatin. A key unanswered question is how developmental cues can direct spatiotemporal chromatin state changes. Here we show that during flower formation in Arabidopsis thaliana, a hormonal cue modulates chromatin and cell state by regulating the association of SWI/SNF chromatin remodelers with target loci. In plants, the positioning and elaboration of flower primordia is controlled by local accumulation of the hormone auxin and by transcriptional regulators called Auxin Response Factors (ARFs) and Aux/IAA proteins. ARFs like ARF5/MONOPTEROS(MP) promote transcription of auxin response genes while Aux/IAA proteins inhibit ARF's transcriptional activity. We show that upon auxin sensing MP recruits two SWI/SNF chromatin remodeling ATPases to overcome a repressive chromatin state at key target loci. Auxin-sensitive Aux/IAA proteins block this recruitment in the absence of the hormone. Presence of at least one of the SWI/SNF ATPases is required to promote the hormone-directed chromatin state changes and flower primordium initiation. Thus, a simple hormone-dependent chromatin switch directs alternative cell fates in inflorescences.

Keywords: Chromatin, flower primordium, Auxin, MP, SWI/SNF chromatin remodeling ATPases

Poster #294. Heat activation and epigenetic silencing in a retrotransposon (Submission 392)
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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 (RdDM) and TEs silencing. Recently we reported that 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 heat activation on ONSEN, we constructed a transgenic plant to analyze the promoter activity of ONSEN by green fluorescent protein (GFP). GFP signals were observed on the transgenic plant in both wild-type and polIV mutant subjected to heat stress. GFP intensity was higher in a polIV mutant compared with that of wild-type.
Transcriptional activity of ONSEN was decreased in several days after heat stress in both wild type and polIV mutant indicating that the silencing is independent of an RdDM mediated pathway.

Keywords: Transposon, Epigenetics, Environmental Stress

**Poster #295. Investigating locus-specific effects in Arabidopsis thaliana RNA silencing pathways** (Submission 416)
Eleanor Walton¹, Louise Jones¹
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RNA silencing is a sequence-specific form of gene regulation that is triggered by double-stranded RNA. In plants it can involve silencing at either the post transcriptional or transcriptional level and is often associated with RNA-directed DNA methylation (RdDM). At the post-transcriptional level, small RNA-containing effector complexes act by cleaving mRNA or by inhibiting translation. Such post-transcriptional gene silencing (PTGS) can become self-maintaining through the action of RNA-dependent RNA polymerase (RDR) enzymes. At the transcriptional level RNA silencing can act to suppress gene expression via the RdDM pathway and/or by directing histone modifications. Although many protein components of these pathways have been identified, the features that identify a sequence as being able to support maintenance of PTGS and/or RdDM are not well understood. This research has aimed to address this by generating and characterizing multiple independent single locus GFP transgenic Arabidopsis lines that have been triggered to undergo silencing by crossing with an amplicon trigger line (AMP243). The ability of these lines to undergo RDR6-dependent PTGS and/or RdDM has been investigated in an attempt to establish a pattern. Additionally epitope-tagged versions of RDR2 and RDR6 have been generated in order to investigate their recruitment to target loci. Amplicon-triggered PTGS occurred in all the independent lines suggesting that RDR6 activity is not influenced by either genome location or locus structure. Despite the production of high levels of sRNAs, not all lines supported RdDM. Results suggest that the ability to undergo RdDM is associated with a complex locus structure rather than genome location and that RdDM is enhanced when targets are in a homozygous state.

Keywords: RNA silencing, RdDM, RDR6-dependent PTGS

**Poster #296. Cell-type specific chromatin accessibility dynamics in the Arabidopsis root.** (Submission 428)
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As transcription factors typically bind naked, histone-free DNA, their activity is directly dependent upon overall chromatin accessibility and remodelling processes across the genome. Consequently, the spatial accessibility of genomic DNA has a direct impact on overall transcriptional regulation processes. As genome-wide chromatin accessibility patterns are expected to vary both between tissues and individual cell types in a manner dependent upon the functional role of each genomic instance, the ability to study chromatin isolated from specifically targeted cell-types would provide a means to investigate cellular chromatin dynamics at an increased level of resolution, free from agglomerative effects arising from whole-tissue study. To this end, we have adopted the INTACT (Isolation of Nuclei Tagged in Specific Cell Types) approach of Henikoff & Deal (2011) and generated a set of lines facilitating the rapid isolation of nuclei from epidermal, cortex, endodermal, stele and root hair cells in the Arabidopsis root. This toolkit is being used to investigate the chromatin dynamics of stress response in the root cells of plants experiencing flooding and drought stress. Cell-type specific chromatin is isolated from INTACT nuclei and partially digested with DNAse I before next-generation sequencing (DNAse-seq). Novel informatic and sequencing methods are being used to facilitate a DNAse-seq method that requires only nanogram quantities of DNA. Chromatin accessibility data obtained from DNAse-seq experiments will be integrated with RNA-seq data in order to identify expression changes in genes whose promoter's fall within regions of remodelled chromatin. It is expected that downstream bioinformatics looking at differential expression/accessibility and regulatory motif prediction will provide insights into the nature of stress responsive genes expressed from regions of remodelled chromatin as well as identify putative regulatory motifs governing chromosomal changes and gene expression.
Keywords: Epigenetics, chromatin, Bioinformatics, Next-generation sequencing, DNase-seq, RNAse-seq, Abiotic stress

**Poster #297. Arabidopsis BPC6 interacts with Polycomb Proteins at PRE-like GAGA DNA-motifs** (Submission 469)
Andreas Hecker¹, Luise H. Brand¹, Sebastian Peter¹, Joachim Kilian², Valerie Gaudin³, Dierk Wanke⁴
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The DNA-binding proteins Pleiohomeotic (PHO) and the GAGA motif binding Trithorax-like (Trl) or Pipsqueak (Psq) are important regulators in Drosophila. They control gene expression via Polycomb repressive complex (PRC) 1 and 2 function at Polycomb repressive DNA-elements (PREs) in a context dependent manner. The equivalent DNA-binding proteins in plants that communicate with either PRC1 or PCR2 are still elusive. Here we show that Arabidopsis thaliana BPC6 interacts with plant PRC1 component LHP1 in the nucleoplasm. BPC6 and its paralog BPC4 are group II BPC proteins that are both characterized by an Alanine-zipper dimerization domain, which is also required for the interaction with LHP1. This BPC6-LHP1 complex functions probably in a larger association with other proteins that include PRC2 components such as VRN2 and possibly MSI1. By DPI-R-ELISA we found that BPC6 is sufficient to recruit LHP1 to GAGA motif containing DNA-motifs. Lhp1-4 bpc4 bpc6 triple mutants display pleiotropic phenotypes, extreme dwarfism and early flowering, which suggests synergistic interaction between LHP1 and group II BPCs. Transcriptome analyses support a synergistic function of plant GAGA-factors and LHP1 to repress homeotic genes, such as FT, AGAMOUS or SHATTERPROOF. Our findings suggest a role of group II BPCs for the recruitment of PRC1 and PRC2 components to PRE-like GAGA-motifs.

Keywords: Polycomb repressive complex, Polycomb repressive element, GAGA motif, Basic Pentacysteine Proteins (BPCs)

**Poster #298. Zinc finger based proteins to control meiotic recombination** (Submission 496)
Martijn Rolloos¹, Frederik Spanhoff¹, Bert van der Zaal¹, Paul Hooykaas¹
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In previous work we proved that genome interrogation via zinc finger based artificial transcription factors (ZF-ATFs) can be successfully applied to screen for novel phenotypic traits in plants. We thus identified a particular transcription factor, designated VP16-HRU, able to induce a 200- to 300-fold increase in the frequency of somatic intrachromosomal homologous recombination (iHR). A deep sequencing strategy allowed for the detection of possibly causal changes in the transcriptome. For at least three genes, ectopic expression led to enhanced iHR frequencies. We are currently investigating the effects of VP16-HRU as well as individual endogenous genes upon meiotic recombination. Apart from that, since recent data provide strong evidence that the local chromatin structure is a prime determinant for the final establishment of a recombination event, we will be investigating whether it will be possible to use ZF-based proteins for modulating genome wide epigenetic marks in plant meiocytes. This will be done by expressing ZF domains fused to protein domains that can be expected to exert an effect upon chromatin structure. By using ZF domains of different complexity, the cognate DNA target site can be chosen to occur with different frequencies within the plant genome. A dedicated collection of gene constructs is currently introduced into Arabidopsis plants in order to get proof of principle for this method.

Keywords: Meiosis, Zinc fingers, recombination

**Metabolism/Biochemistry**

**Poster/Talk #299. The metabolic target of pyrophosphate, a mysterious player in plant metabolism, identified** (Submission 14)
Ali Ferjani¹, Kensuke Kawade², Akira Oikawa³, Mariko Asaoka⁴, Kazuki Takahashi¹, Masayoshi Maeshima⁴, Masami Yokota Hirai², Kazuki Saito³, Hirokazu Tsukaya⁴
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Following germination, oil-seed plants rely on triacylglycerol reserves to sustain growth during a heterotrophic period that requires b-oxidation, the glyoxylate cycle, and gluconeogenesis activities to convert triacylglycerol to sucrose. In addition, macromolecular syntheses in proliferating tissues consumes huge amounts of ATP, often hydrolyzed to AMP plus PPI. In Arabidopsis, the proton-pumping vacuolar pyrophosphatase (H+-PPase) uses the energy from PPI hydrolysis to acidify the vacuole. The Arabidopsis fugu5 mutants, lacking the AVP1 H+-PPase, failed to sustain postgerminative heterotrophic growth, which recovered upon sucrose supply, or specific PPI removal by the cytosolic IPP1 from yeast in AVP1pro:IPP1 transgenic lines. This demonstrated that the major function of H+-PPase in seedling development is the removal of inhibitory PPI rather than proton pumping. Following quantification of sucrose and PPI amounts in the wild-type versus fugu5, we concluded that it is gluconeogenesis that is inhibited by elevated level of PPI, but the mechanism or targets of PPI inhibition remained unclear. Here, our profiling of major metabolites that occur during triacylglycerol mobilization showed that the amounts of Glu-1-P and UDP-Glc were 2.0- and 0.5-fold in fugu5 versus the wild type, while other intermediate metabolites were unaffected. This indicated that UDP-Glucosyl Pyrophosphorylase (UGPase) is specifically inhibited by cytosolic PPI accumulation in fugu5. Importantly, the above metabolic defects were reversed in AVP1pro:IPP1, where Glc-1-P and UDP-Glc were 1.0- and > 2.0-fold compared to the wild type. Thus, given the readily reversible nature of the Glc-1-P/UDP-Glc reaction, the metabolic target of PPI overaccumulation in fugu5 is UGPase.

Keywords: Storage oilseed reserves, Pyrophosphate (PPI), gluconeogenesis

**Poster #300. Identification of an activation-tagged mutant reveals a role of GLABRA2 in anthocyanin synthesis in Arabidopsis (Submission 18)**
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In Arabidopsis, anthocyanin synthesis is controlled by several transcription factors including R2R3 MYB transcription factors PRODUCTION OF ANTHOCYANIN PIGMENT 1 (PAP1), PAP2, MYB113 and MYB114, bHLH transcription factors GLABRA3 (GL3), ENHANCER OF GLABRA3 (EGL3) and TRANSPARENT TESTA8 (TT8), and a WD40-repeat protein TRANSPARENT TESTA GLABRA1 (TTG1). PAP1/PAP2/MYB113/MYB114, GL3/EGL3/TT8 and TTG1 form a MYB-bHLH-WD40 (MBW) transcription factor activator complex to activate the transcription of late biosynthesis genes in the flavonoid pathway, leading to the production of anthocyanins. A similar MBW complex regulates trichome and root hair formation in Arabidopsis by activating the transcription of GLABRA2 (GL2). The same MBW complex also induces the expression of single-repeat R3 MYB genes. Available evidence suggests that R3 MYBs can also regulate anthocyanin synthesis. However, the role of GL2 in anthocyanin synthesis remains unknown. From an activation-tagged mutagenized population of Arabidopsis in gpa1-2 mutant background, we identified a dominant mutant with less anthocyanins. By using TAIL-PCR, we found the T-DNA was inserted in chromosome 1 at a position that is 1,886bp upstream the start codon of GL2 and 1698bp upstream of the start codon of GC5, with the four outward facing 35S enhancers oriented toward GL2. RT-PCR analysis revealed that the expression of GL2, but not GC5, was elevated, indicating that GL2 was tagged. Thus the mutant was named gl2-1D. By crossing gl2-1D with Col wild type plant and examining phenotypes of F1 and F2 plants, we found that the phenotype of gl2-1D was caused by a single T-DNA insertion and is independent of the gpa1-2 mutation. Consistent with the view that GL2 acts as a negative regulator of anthocyanin synthesis, gl2-1D seedlings are hyposensitive whereas gl2-3 seedlings are hypersensitive to sucrose-induced anthocyanin synthesis. When recruit to the promoter of a LexA-GaL4-GUS reporter gene by a Gal4 DNA binding domain (GD) in a protoplast transient transfection assay, GL2 repressed the activation of the reporter gene by a co-transfected LD-VP16 transcription activator, suggesting that GL2 is a transcriptional repressor. RT-PCR results showed that expression of PAP2 is reduced in gl2-1D and elevated in gl2-3, suggesting that PAP2 is likely a target gene of GL2. In summary, our results suggest that GL2 is a transcriptional repressor involved in the regulation of anthocyanin synthesis in Arabidopsis.
Keywords: Anthocyanin, Arabidopsis, GLABRA2, Transcription factor

Poster #301. N-linked glycosylation of AtVSR1 is essential for vacuolar protein sorting in Arabidopsis (Submission 46)
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The Arabidopsis vacuolar sorting receptors (AtVSRs) mediate sorting of soluble proteins to vacuoles in the secretory pathway [1,2]. The AtVSRs are post-translationally modified by the attachment of N-glycans, but the functional significance of this modification is unknown. Here we report on the role of glycosylation on AtVSR1 stability and localization, and on its vacuolar sorting activity in Arabidopsis protoplasts. AtVSR1 harbors three predicted complex type N-glycans, located in the N-terminal `PA domain`, central region and C-terminal EGF-repeat domain, respectively [3]. We have demonstrated that 1) the N-glycans do not affect the AtVSR1 protein targeting to the prevacuolar compartments (PVCs) and its vacuolar degradation; and 2) N-glycosylation alters the AtVSR1 binding affinity to cargo proteins and affects the transport of cargo into the vacuole. Therefore, N-glycans of AtVSR1 play a critical role for its function as a vacuolar sorting receptor. Supported by grants from the Research Grants Council of Hong Kong (CUHK466011, 465112 and 466613, CUHK2/CRF/11G and AoE/M-05/12).

References:

Keywords: vacuolar sorting receptor, N-glycosylation, vacuolar protein, Arabidopsis

Poster/Talk #302. The Arabidopsis Mediator subunit MED16 regulates iron homeostasis by associating with EIN3/EIL1 through subunit MED25 (Submission 71)
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Iron is an essential micronutrient for plants and animals, and plants are a major source of iron for humans. Therefore, understanding the regulation of iron homeostasis in plants is critical. We identified a T-DNA insertion mutant, yellow and sensitive to iron deficiency 1 (yid1), that was hypersensitive to iron deficiency. YID1 encodes the Arabidopsis Mediator complex subunit MED16. YID1/MED16 interacts with another Mediator subunit, MED25 both in vitro and in vivo. MED25 is involved in regulation of iron homeostasis by interacting with EIN3 and EIL1, two transcription factors in ethylene signaling that are associated with regulation of iron homeostasis. Furthermore, the transcriptome in yid1 and med25 mutants was significantly affected by iron deficiency. In particular, the transcripts of FIT, IRT1 and FRO2 were reduced during iron deficiency in yid1 and med25 mutants when compared with those in the wild type. The finding that YID1/MED16 and MED25 positively regulate iron homeostasis in Arabidopsis facilitates to understand the complex networks for iron homeostasis regulation in plants.

Keywords: yid1, Plant iron homeostasis, Mediator complex, MED16, MED25

Poster/Talk #303. Stress-responsive aldehyde dehydrogenase 3H1: Identification of amino acid residues critical for cofactor specificity and thiol regulation (Submission 83)
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Lipid peroxidation is a consequence of environmental stress in plants and leads to the accumulation of highly...
reactive and cytotoxic aldehydes. One of the processes to detoxify these aldehydes and withstand the associated damage is their irreversible oxidation to the corresponding carboxylic acids. This reaction is catalyzed by NAD(P)⁺-dependent ALDHs (aldehyde dehydrogenases, E.C 1.2.1.3) which constitute a large evolutionary conserved superfamily of enzymes. The genome of Arabidopsis thaliana comprises 16 genes encoding ALDHs belonging to ten families. Members of families 3 and 7 are involved in stress protection. The cofactor-binding site of the family 3, NAD⁺-dependent and stress-responsive ALDH3H1 was analyzed to unravel structural features determining cofactor-specificity. The amino acid E149 occupies a central position in the cofactor-binding cleft, and its carboxylate group coordinates the 2' and 3'-hydroxyl groups of the adenosyl ribose ring of NAD⁺ while it repels the 2' phosphate moiety of NADP⁺. Substitution of E149 to Q, D, N or T altered the interaction with NAD⁺ and rendered the enzyme capable of using NADP⁺. This change is attributed to a weaker steric hindrance or/and an elimination of the electrostatic repulsion of the 2'-phosphate of NADP⁺. Simultaneous mutations of E149 and I200 improved the catalytic efficiency with NADP⁺. The double mutant ALDH3H1E149T/I200V showed a better catalysis with NADP⁺. Subsequently a third mutation was performed by replacing V178 by R to generate a "closed" cofactor binding site. The cofactor specificity was shifted even further in favor of NADP⁺, as the triple mutant ALDH3H1E149T/V178R/I200V uses NADP⁺ with almost 7-fold higher catalytic efficiency compared to NAD⁺. Homology modeling and engineering the coenzyme-binding environment elucidated that amino acids occupying positions 149, 178 and 200 are relevant for the determination of cofactor affinity in class 3 ALDHs.

ALDH3H1 and its chloroplastic homologue ALDH3I1 are subject to thiol regulation. Oxidation and S-nitrosylation lead to an increase of activity simultaneously to a disulfide-bridge mediated homodimerization only in case of oxidation. Mutagenesis studies and specific chemical labeling of the active site cysteine demonstrated that the latter is the target of post-translational modifications.

Keywords: aldehyde dehydrogenase, abiotic stress, cofactor specificity, thiol regulation, chemical labeling

Poster/Talk #304. Pollen-specific flavonol biosynthesis in Arabidopsis thaliana: identifications of the key enzyme UGT33 and the tailored flavonol structures (Submission 134)
Keiko Yonekura-Sakakibara¹, Ryo Nakabayashi¹, Satoko Sugawara¹, Takayuki Tohge¹, Takuya Ito¹, Misuzu Koyanagi², Mariko Kitajima², Hiromitsu Takayama², Kazuki Saito³
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Flavonol 3-O-diglucosides with a 1-2 interglycosidic linkage are representative pollen-specific flavonoids widely distributed in plants, but their biosynthetic genes and physiological roles are not well understood. We analyzed the flavonoid profiles of Arabidopsis floral organs (pistils, stamens, petals and calyxes) and flowers of wild-type and male sterility 1 (ms1) mutants, which are defective in normal development of pollen and tapetum and determined the precise structures of flavonol 3-O-diglucosides accumulated in Arabidopsis pollen. Based on microarray data using wild-type and ms1 mutants, gene expression patterns in various organs, and phylogenetic analysis of UGTs, UGT33 is found to be a key modification enzyme for determining pollen-specific flavonol structure. Pollen-specific flavonol 3-O-diglucosides were absent from two independent ugt33 knockout mutants. Transgenic ugt33 mutant lines transformed with the genomic UGT33 gene had the same flavonoid profile as wild-type plants. Recombinant UGT33 protein converted kaempferol 3-O-glucoside to kaempferol 3-O-glucosyl-(1-2)-glucoside. UGT33 recognized 3-O-glycosylated/galactosylated anthocyanins/flavonols but not 3,5- or 3,7-diglycosylated flavonoids, and prefers UDP-glucose, indicating that UGT33 encodes flavonoid 3-O-glucosyltransferase. A UGT33-ss-glucuronidase fusion showed that UGT33 was localized in tapetum cells and microspores of developing anthers.

Keywords: glucosyltransferase, glycosyltransferase, flavonoid, pollen

Poster/Talk #305. An intermediate cleavage peptidase modifies enzyme N-termini, alters protein stability and influences serine metabolism in Arabidopsis mitochondria (Submission 145)
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Mitochondrial-targeted precursor proteins containing peptide signals are cleaved after import by the mitochondrial processing peptidase. In yeast and human the intermediate cleavage peptidase 55 (ICP55) plays a role in further processing and stabilising of mitochondria proteins by removal of single amino acids. We have investigated the role of a putative mitochondrial metallopeptidase (At1g09300) from the model plant Arabidopsis which has sequence similarity to yeast ICP55. This protein was found in Arabidopsis mitochondria by mass spectrometry and its function studied in a T-DNA insertion line (icp55). Monitoring of the N-terminal peptides of a series of proteins showed that plant ICP55 is responsible for removal of single amino acids that generate the -3 arginine processing motif of selected mitochondrial proteins, as well as removal of single amino acids from other proteins to generate unique non-conserved arginine motifs that are specific to plant mitochondrial proteins. Icp55 showed faster mitochondrial protein degradation rates than mitochondrial samples isolated from wildtype for a range of proteins. Icp55 did not show any gross phenotypic difference in growth under short and long days, elevated temperature or light. The lower protein stability in isolated mitochondria and the lack of processing of target proteins in icp55 can be complemented by transformation with the full-length ICP55 cDNA (At1g09300) driven by the 35S promoter. Analysis of in vitro degradation rates and protein turnover rates in vivo of specific proteins and analysis of metabolite pool sizes indicated that SHMT and serine metabolism were consistently affected in icp55. The maturation of serine hydromethyltransferase (SHMT) by ICP55 is unusual as it involves breaking an N-terminal diserine (S|S) which is not known as an ICP55 substrate in other organisms and is typically considered a stabilising rather than a destabilising sequence. Possible networks controlling protein stability/degradation involving ICP55 and other mitochondrial proteases are discussed.

Keywords: Mitochondrial protein stability, Cleavage peptidase, Serine metabolism

**Poster #306. Promoter Analysis of Glycyrrhizin Biosynthetic Genes Using Transgenic Arabidopsis thaliana** (Submission 156)
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Biosynthesis of plant secondary metabolites (also called specialized metabolites) is strictly regulated in a spatial and temporal manner and influenced by external and/or internal stimuli. This regulation can often be explained by the transcriptional control of corresponding biosynthetic genes by transcription factors (TFs). Some TFs have been characterized as critical regulators in flavonoid and alkaloid biosynthetic pathways. On the other hand, regulatory mechanism underlying triterpenoid biosynthesis is still largely unknown. Triterpenoids are a group of specialized metabolites, derived from a common precursor 2,3-oxidosqualene, including various bioactive compounds such as ginsenosides, glycyrrhizin and oleanolic acid. In this study, we focused on glycyrrhizin biosynthetic pathway in Glycyrrhiza uralensis (licorice). Glycyrrhizin shows a wide range of pharmaceutical effects such as hepatoprotective, anti-allergy and anti-viruses, and also shows 150-200 times stronger sweetness than sucrose. Its biosynthesis involves the cyclization of 2,3-oxidosqualene catalyzed by b-aminyr synthase (bAS) and subsequent multistep oxidation at C-11 and C-30 positions of b-aminyr catalyzed by two cytochrome P450 monoxygenases, CYP88D6 and CYP72A154, respectively.

To analyze the promoter activities of these biosynthetic genes, we isolated about 2 kb upstream sequences from the translation start site of bAS and CYP88D6 from two different licorice strains, high-glycyrrhizin production (HGLP) and low-glycyrrhizin production (LGLP) strains. In order to visualize their expression patterns in a whole plant, we generated transgenic Arabidopsis lines carrying promoter::GUS fusions for bAS and CYP88D6. Consistent with the expression patterns of corresponding genes in licorice as revealed by RT-PCR analysis, both promoters conferred root-specific GUS expression, but zone-specific difference was observed between bAS and CYP88D6 gene promoters. Comparison of promoter sequences obtained from HGLP and LGLP strains revealed some polymorphism between these strains, however no clear correlation between polymorphisms and promoter activities were detected. These results suggest that the different expression levels of bAS and CYP88D6 in HGLP and LGLP strains could be attributed to the difference in the expression levels of transcription factor(s) regulating bAS and CYP88D6.

Keywords: triterpenoid biosynthesis, glycyrrhizin, transcriptional regulation
Poster #307. Reduce, Reuse, Recycle: How do plants deal with free xylose? (Submission 204)
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In plants, a range of free monosaccharides such as galactose, arabinose, and fucose are released from the cell wall due to restructuring, expansion, pathogen infection and glycoprotein turnover. These free sugars are taken up by the cell and recycled into nucleotide sugars through well characterized salvage pathways. However, recycling of xylose is less clear. It has been proposed that free xylose is either salvaged into the nucleotide sugar UDP-Xyl or catabolized via the pentose phosphate pathway. Recently we identified the initial step in xylose catabolism, xylose isomerase, (AT5G57655) in Golgi enriched fractions of Arabidopsis. The xylose isomerase (XI) catalyzes the isomerization 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 biochemical and functional role of this enzyme in plants. Transiently expressed xylose isomerase fused to YFP confirmed an endoplasmic reticulum (ER) association, likely with the active site inside the lumen. Furthermore, yeast (Saccharomyces cerevisiae) ectopically expressing the Arabidopsis XI gene (AtXI) are capable of using xylose as the carbon source and confirms its biochemical function as a xylose isomerase. The biochemical activity of AtXI has a preference toward the production of xylose (compared to xylulose) which potentially explains its functional compartmentalization in the ER lumen. Knocking down XI by virus induced gene silencing (VIGs) in tobacco showed retarded growth. This slow growth phenotype also displayed in the seedlings of a T-DNA insertion line of XI. In combination with stable isotope feeding experiments, we hope

Keywords: xylose isomerase, xylose catabolism, pentose phosphate pathway

Talk #308. Unsaturation of Very-Long-Chain Ceramides Protects Plant from Hypoxia in Arabidopsis (Submission 236)
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Lipid remodeling is crucial for hypoxic tolerance in animals, while little is known about the hypoxia-induced lipid dynamics in plant cells. Here we performed a mass spectrometry-based analysis to survey the lipid profiles of Arabidopsis rosettes under various hypoxia conditions. We observed that hypoxia caused a significant increase in total amounts of phosphatidylserine, phosphatidic acid and oxylipins, but a decrease in phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Particularly, significant gains in the polyunsaturated molecular species of PC, PE and phosphatidylinositol, and losses in their saturated and mono-unsaturated species were evident during hypoxia. Moreover, hypoxia leaded to a remarkable elevation of ceramides and hydroxyceramides. Knockout mutants of ceramide synthase genes LOH1, LOH2 and LOH3 showed enhanced sensitivity to dark submergence (DS), but more resistance to submergence under light than wild type. Consistently, levels of unsaturated species (22:1, 24:1 and 26:1) of ceramides were predominantly declined in the loh1, loh2 and loh3 mutants under DS. Evidence that C24:1-ceramide interacted with recombinant CTR1 protein in vitro, enhanced ER-to-nucleus translocation of EIN2-GFP and stabilization of EIN3-GFP in vivo, suggests a role of ceramides in modulating ethylene signaling. The DS-sensitive phenotypes of loh mutants were rescued by a ctr1-1 mutation. Thus, our findings demonstrate that unsaturation of very-long-chain ceramides is a protective strategy for hypoxic tolerance in Arabidopsis.

Keywords: ceramides, CTR1, ethylene signaling, fatty acyl unsaturation, hypoxic tolerance

Poster #309. Alternative translation of ATP sulfurylase 2 allows dual localization of sulfate assimilation in chloroplasts and cytosol in Arabidopsis thaliana (Submission 238)
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Sulfur is an essential nutrient for plant growth and is found in cellular components such as amino acids, sulfolipids and various key metabolites involved in biotic and abiotic responses. The first step of the sulfate assimilation pathway is catalyzed by ATP sulfurylase. Arabidopsis thaliana has four ATP sulfurylase genes (ATPS1 to 4).
Although all four ATPS genes have predicted transit peptide for plastid targeting, ATPS activity can be found in both plastids and cytosol.

Sequence analysis suggests that ATPS2 could produce a cytosolic isoform as its protein coding region may contain alternative translation initiation sites which correspond to Met-52 and Met-58 in its transit peptide region. Based on this hypothesis, we investigated whether the translation can be initiated from these alternative sites. Using dual luciferase reporter genes, we constructed a tandem fusion gene of Met-1-Renilla Lucerase (M1-Rluc) and Met-52/Met-58-Firefly Lucerase (M52M58-FLuc), which allows transcription of a polycistronic mRNA and subsequent translation of a single transcription unit into two different luciferases. The fusion gene construct was introduced into Arabidopsis protoplasts, and luciferase activities were monitored to determine the presence of translational products, M1-Rluc and M52M58-FLuc. We detected a firefly luciferase activity in the protoplasts, suggesting that M52M58-FLuc is produced and that an alternative translation initiation site for ATPS2 should be functional for translating the cytosolic ATPS isoform. Point mutations of various Met residues identify Met52 as the alternative site for producing cytosolic ATPS2 isoform. Transient expression of ATPS2-GFP fusion protein supported this result. Moreover, translation efficiency of ATPS2 at Met52 is not modulated by sulfate conditions although the abundance of chloroplast isoforms (ATPS1, 3 and 4) is reduced under sulfur deficient condition. These results indicate that ATPS2 mRNA can be translated alternatively in two different isoforms, thus allowing a dual localization for ATPS2 enzyme.

Keywords: Sulfate assimilation, ATP Sulfurylase, Alternative translation, Dual localization

Poster/Talk #310. A selective inhibitor of jasmonate signaling targets the adenylate-forming enzyme JAR1 in Arabidopsis thaliana (Submission 242)

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Jasmonates are lipid-derived plant hormones that regulate plant defenses and numerous developmental processes. Although the biosynthesis and molecular function of the most active form of the hormone, (+)-7-isojasmonoyl-L-isoleucine (JA-Ile), have been unraveled, it remains poorly understood how the diversity of bioactive jasmonates regulates such multitude of plant responses. Bioactive analogs have been used as chemical tools to interrogate the diverse and dynamic processes of jasmonate action. By contrast, small molecules impairing jasmonate functions are currently unknown. Here, we report on jarin-1 as the first small molecule inhibitor of jasmonate responses that was identified in a chemical screen using Arabidopsis thaliana. Jarin-1 selectively impairs the activity of jasmonoyl-L-isoleucine synthetase (JAR1), thereby preventing the synthesis of the active hormone, JA-Ile, whereas closely related enzymes are not affected. Thus, jarin-1 may serve as useful chemical tool in search for missing regulatory components and further dissection of the complex jasmonate signaling networks.

Keywords: Bioactive small molecules, Chemical biology, Chemical genetics, Chemical screening, Jasmonoyl-L-isoleucine, Jasmonate metabolism, Jasmonate signaling, Plant hormone metabolism, Plant defense

Poster #311. Identification of ADP-ribosyl cyclase in Arabidopsis thaliana (Submission 251)

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In animals, ADP-ribosyl cyclase (ADPR cyclase) is a multifunctional enzyme that catalyses nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP) into two important second messengers, cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP), respectively. cADPR targets the ryanodine receptor (RyR) present in the sarco/endoplasmic reticulum (SR/ER) and acts as a potent calcium mobiliser in the cell. cADPR elevates cytosolic free Ca^{2+}[Ca^{2+}]_{cyt} in plants and plays a central role in signal transduction pathways evoked by the drought and stress hormone, abscisic acid (ABA). Despite evidence for the action of cADPR in plants, no predicted proteins with significant similarity to the
known ADPR cyclases have been reported in any plant genome database, suggesting that proteins with very low homology or a unique mechanism for cADPR synthesis must exist in plants. Therefore, the main objective of the project is identification of the functional part of the ADPR cyclase enzyme in Arabidopsis, responsible for cADPR synthesis. As the activity of this protein is very low in Arabidopsis, we used a pharmacological approach with agonists (NaCl, H2O2, Nitric Oxide, and Cold Water) and antagonists (Nicotinamide, 8-Br-cADPR, Mg2+) to identify possible regulators of ADPR cyclase through [Ca2+]cyt measurements and found that nitric oxide (NO) is a potential candidate for increasing the activity of ADPR cyclase. NO can increase the activity of the enzyme 3-fold and nicotinamide inhibits the enzyme activity. cADPR-mediated [Ca2+]cyt oscillations are involved in the circadian clock. CIRCadian CLOCK ASSOCIATED1 (cca1) acts as a repressor of ADPR cyclase activity in Arabidopsis, consistent with the circadian regulation of [Ca2+]cyt. We are proceeding to purification of the protein.

Keywords: Cyclic ADP-Ribose, Circadian Clock, Nitric Oxide, Nicotinamide

Poster #312. Structure-function analysis of Arabidopsis feruloyl-CoA 6'-hydroxylase 2 (Submission 255)
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In Arabidopsis thaliana genome there are several genes encoding members of the 2-oxoglutarate (2OG)- and Fe(II)-dependent dioxygenase (2OGD) family. The 2OGD is the second largest enzyme family in plants and its members are involved in the biosynthetic pathways of various secondary metabolites, including coumarins. It was shown previously that coumarins (predominantly scopolin and scopoletin) occur in Arabidopsis roots. Scopoletin and its glucoside scopolin are natural compounds, which are synthesized in plants as a defence mechanism against various environmental stresses. They have significant biological activities and numerous have found medical application. The feruloyl-CoA 6'-hydroxylase 1 and 2 (F6'H1 and F6'H2 respectively) were shown to catalyze the ortho-hydroxylation of feruloyl-CoA, which is an important step in scopoletin biosynthesis. It was shown in our group that a significant natural variation in the accumulation of scopolin and scopoletin is present among 28 Arabidopsis accessions. Using real-time qPCR, we have tested the expression levels of the F6'H1 and F6'H2 genes in two Arabidopsis accessions (Col-0 and Est-1), which differed in scopoletin accumulation. In a higher accumulator (Est-1), the expression level of both genes was higher, in particular the expression of F6'H1. After re-sequencing we did not detect any polymorphism in the coding sequences of F6'H1 but instead we identified two insertions in its promotor region in Est-1. Interestingly, we also detected two SNPs in the N-terminus of F6'H2 leading to an arginine to proline substitution in Est-1. In order to check the impact of this substitution on the F6'H2 activity, we performed enzymatic activities measurements by using heterologous expression in Escherichia coli and transient expression in Nicotiana benthamiana leaves followed by scopoletin extraction and UPLC/MS analysis. We detected a higher in vitro enzymatic activity of Col-0 F6'H2 in E. coli, which was in agreement with a higher scopoletin accumulation obtained in N. benthamiana leaves transformed with pBin-3SS-Col-0-F6'H2. Importantly, molecular modelling of F6'H2 showed differences in Col-0 and Est-1 protein structures, which could explain slower enzymatic reaction rate of Est-1. We detected more ligation points for the substrate (feruloyl-CoA) in Est-1, which could lead to a longer remaining time inside the enzyme pocket and as a result to a lower efficiency of Est-1 F6'H2 compared to Col-0.

Keywords: coumarins, enzyme activity, F6'H2, natural variation, scopoletin, secondary metabolism, structure-function analysis

Poster #313. Comparison of the Starch Binding Module of the SnRK1 Akinb and Akinbg subunits (Submission 281)
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Plants are highly specialized organisms that confront and adapt to different environmental and nutritional conditions. Under these circumstances, they trigger signaling pathways to orchestrate several responses. SnRK1 is a heterotrimeric complex with kinase activity that directly modify cell metabolism and gene expression in
response to unfavorable energetic conditions. The complex consists of a a-catalytic subunit and two regulatory b
and g subunits. In Arabidopsis thaliana, the catalytic subunits are encoded by akin10 and akin11. Overexpression
and null akin10 Arabidopsis plants indicated a direct role of SnRK1 in starch metabolism. SnRK1 regulates the
expression of a-amylase and ADP-glucose pyrophosphorylase and indirectly regulates the activity of ADP-
glucose pyrophosphorylase. Akinb1, Akinb2 and AKINbg, contain a N-terminal Starch binding domain (SBD)
which allows the association with the starch. Experiments performed in our laboratory, showed that point
mutations in amino acids directly involved in the interaction of AKINbg with the carbohydrate, modified very little
its affinity, whereas proteins lacking the complete SBD domain lost the ability to bind the starch. In this work we
compared the structure of the SBD from the b- and AKINbg subunits with the structure of the GBD from AMPKb1
to identify important regions for carbohydrate binding. In addition, we evaluated the capacity of the recombinant
proteins to bind amylose/amylopectin mixtures as well as starch granules from different sources. We discussed
the putative function of the subunits according to their subcellular localization and to their presence in the different
complexes.
This work was supported by DGAPA (IN211513), PAIP 5000-9126.

Keywords: SnRK1 heterotrimeric complex, Starch Binding Domain (SBD), AKIN betagamma subunit

**Poster #314. ARF7 regulates the expression of BR-inactivating gene BAS1 in Arabidopsis** (Submission
356)
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Brassinosteroid (BR) is a steroidal plant hormone that regulates many aspects of physiological and
developmental responses in plant. Endogenous BR levels are strictly controlled by homeostatic regulation through
biosynthesis and inactivation/degradation. Although many BR metabolic genes mediating biosynthesis and
inactivation have been identified and their expression is regulated by BZR1-mediated feedback loop, it remains
largely unknown how their expression is initiated by specific cellular signals. Arabidopsis BAS1 (CYP734A1)
inactivates the biologically active BRs via C-26 hydroxylation. Here we show that BZR1 and ARF7 antagonistically
regulate the expression of BAS1 gene in Arabidopsis. Expression of BAS1 is increased in the arf7 and arf7arf19
mutant. Consistently, it revealed that the endogenous level of active BR, castasterone, is greatly reduced in those
mutants. Histochemical analysis of BAS1pro::GUS plants showed that two E-box cis-elements of BAS1 promoter
are important to BR-regulated BAS1 expression. Both BZR1 and ARF7 bound to those E-box motifs of BAS1
promoter. Moreover, we demonstrate that ARF7 directly interacts with BZR1 in vivo and in vitro, which inhibits
their binding to BAS1 promoter. Taken together, our results indicate that ARF7 regulates the expression of BR-
inactivating gene BAS1 through competition with BZR1, resulting in the increase of endogenous active BR level.

Keywords: Brassinosteroids, BR homeostasis, BAS1, Auxin, ARF

**Poster #315. CER2-LIKE proteins modify the activities of specific condensing enzymes to make
exceptionally long acyl-lipids of the cuticle and pollen coat** (Submission 380)
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The extension of fatty acids beyond 28 carbons is a unique process in plant metabolism in that it requires
distinct biochemical machinery, and elongation products are used for the synthesis of specialized metabolites.
C30-C34 acyl-lipids make up the bulk of cuticular wax, a surface layer that restricts transpirational water loss, and
are also components of the pollen coat required for pollen hydration. The core elongase complex that synthesizes
very-long-chain fatty acids (VLCFAs; C20-C34) consists of four proteins. Condensing enzymes catalyze the first
elongase reaction and determine the chain length of fatty acids accepted and produced by the complex. While
necessary for the elongation of all VLCFAs, identified condensing enzymes cannot efficiently produce VLCFAs
longer than C28.

The cer2 mutant of Arabidopsis thaliana has a severe wax-deficient phenotype and specifically lacks waxes
longer than C28, but the CER2 protein has no homology to condensing enzymes. Heterologous expression in
yeast has demonstrated that CER2 can modify the chain-length specificity of a condensing enzyme, CER6, from
C28 to C30. We identified two CER2 homologs in Arabidopsis thaliana. Our present investigation of the specificities and physiological roles of this protein family will improve our understanding of their function, by answering three questions: (1) Do CER2-LIKE proteins function only with CER6, or can they modify the activities of other condensing enzymes? (2) Do different CER2-LIKE proteins have different effects on the substrate specificity of the same condensing enzyme? (3) Are CER2-LIKEs involved in metabolic pathways other than wax biosynthesis?

Keywords: lipids, cuticle, very-long-chain fatty acids

Light and Plant Growth

Talk #316. FRS12 and 7 are transcriptional repressors involved in the regulation of circadian growth in Arabidopsis thaliana (Submission 25)
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Plants must efficiently anticipate diurnal oscillation to control essential rhythmic processes. Maximal plant growth, for example, occurs at the end of the night and is controlled by the endogenous clock system and the perception of the light environment. Here, we describe the transcription factors FAR1 RELATED SEQUENCE 7 (FRS7) and 12 (FRS12) that we initially identified as physically interacting with the NINJA-TPL co-repressor complex. Interactome analysis with tandem affinity purification-MS with FRS12 as bait revealed that it integrates into a transcriptional repressor complex composed of FRS7 and AT-hook motif nuclear-localized proteins (AHLs). Arabidopsis lines presenting altered expression of FRS7 and FRS12 show different red light hypocotyl elongation phenotypes, suggesting the implication of FRS7/12 in red light signalling. RNA-Seq analysis revealed that FRS12 overexpression leads to repression of genes involved in red light signalling and circadian rhythm, in particular Phytochrome Interactor Factor 4 (PIF4), one of the transcription factors promoting plant growth at the early evening. ChiP-Seq analysis with FRS12 as bait confirmed the implication of this protein in the control of red light signalling and circadian rhythm and identified PIF4 among its direct target genes. Expression of the FRS7/12 complex is diurnally regulated and peaks at night, which is negatively correlated with the expression of a large portion of its target genes, including PIF4. Therefore, we postulate that the FRS7/12 protein complex constitutes a novel light regulated machinery that controls several aspects of circadian regulation of plant growth.

Keywords: PIF, FRS, circadian rhythm, red light signalling, growth

Poster #317. Chloroplast membrane protein CPP1 is involved in stabilization of light-dependent protochlorophyllide oxidoreductase. (Submission 51)
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Angiosperms require light for chlorophyll biosynthesis, because one reaction in the pathway, the reduction of protochlorophyllide (Pchlide) to chlorophyllide, is catalyzed by the light-dependent protochlorophyllide oxidoreductase (POR). Here we report that chaperone-like protein of POR 1 (CPP1), an essential protein for chloroplast development, plays a role in the regulation of POR stability and function. CPP1 contains a J-like domain and three transmembrane domains and is localized in the thylakoid and envelope membranes, and interacts with POR isoforms in chloroplasts. CPP1 can stabilize POR proteins with its "holdase" chaperone activity. CPP1 deficiency results in diminished POR protein accumulation and defective chlorophyll synthesis, leading to photobleaching and growth inhibition of plants under light conditions. CPP1 depletion also causes reduced POR accumulation in etioplasts of dark-grown plants and as a result impairs the formation of prolamellar bodies, which subsequently affects chloroplast biogenesis upon illumination. Furthermore, in cyanobacteria, the CPP1 homolog critically regulates POR accumulation and chlorophyll synthesis under high light conditions, in which the dark-operative Pchlide oxidoreductase (DPOR) is repressed by its oxygen sensitivity. These findings
and the ubiquitous presence of CPP1 in oxygenic photosynthetic organisms suggest the conserved nature of CPP1 function in the regulation of POR.

Keywords: Protochlorophyllide oxidoreductase, chaperone, etioplast, photobleaching, CPP1

Poster/Talk #318. Ribosome Abundance and Protein Turnover are Negatively Linked to Biomass Accumulation in Arabidopsis in a Stable Diurnal Growth Regime (Submission 56)
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Ribosome production, protein synthesis and turnover require a huge investment of energy and resources. The aim of this work is to investigate whether ribosome production, protein synthesis and turnover could be significant factors in determining plant growth rates. qRT-PCR analysis of rRNA in extracts spiked with eight artificial RNAs was used for absolute quantification of ribosomes. When we used this approach to quantify ribosomes in 20 Arabidopsis accessions which have different growth rates, we unexpectedly found that ribosome abundance was negatively correlated with biomass specifically at the end of the night (EN) (R = -0.63). We next asked if the higher biomass production in large accessions is due to more efficient use of ribosomes. The percentage of ribosomes engaged in protein synthesis at ED and EN was estimated by polysome loading analysis. Polysome loading was similar, or even slightly higher, in small accessions than large accessions. Consequently, ribosome abundance in polysomes at EN was highly negatively correlated with biomass (R = -0.87). Further, the modelled rates of night-time and daily (24 h) protein synthesis were substantially higher in small accessions than in large accessions. The total protein content did not differ significantly between small and large accessions. We reasoned that small accessions may have a higher rate of protein turnover, which competes with growth. To test this idea, a new method was developed to measure the global rates of protein synthesis and turnover in intact plants. Soil-grown Arabidopsis plants were labelled with 13CO2 for an entire 24h cycle, starting just before dawn, and were then transferred to 12CO2 for a 4-day chase. The labelling kinetics of free amino acids and of amino acids that were released from protein by chemical hydrolysis were measured by GC-MS, and used to calculate absolute protein synthesis rates. Many amino acids showed slow and incomplete enrichment; ala and ser were selected for the calculations because they showed rapid labelling up to high enrichment (>75%) and rapid and complete loss of label in the chase. The measured rate of protein synthesis correlated positively with ribosome abundance at EN (R= 0.70) but not at ED. Most importantly, a negative correlation was found between the protein turnover rate and relative growth rate (R= -0.88). These results point to a basic trade-off between protein turnover and maximisation of growth rate.

Keywords: Growth, Ribosome, Protein Turnover

Poster #319. Phytochrome B and the Circadian Clock modulate Glyphosate Resistance through regulating the Shikimate Pathway (Submission 70)
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The shikimate pathway plays a key role in the biosynthesis of essential amino acids such as phenylalanine, tryptophan and tyrosine, as well as vitamins. In addition, it has been subject to increasing attention since it contains the sole target enzyme for the herbicide glyphosate, 5-Enolpyruvyl Shikimate 3-Phosphate Synthase. Widespread glyphosate use in genetically engineered glyphosate-resistant crops causes high selection pressure on weeds and has resulted in weed population shifts and the evolution of over 20 resistant weed species (http://www.weedscience.org). However, the mechanisms of resistance of most of these species, while not well characterized, are believed to be mainly due to alterations of the target enzyme. Here we demonstrate the regulatory roles of phyB signaling and the circadian clock mechanisms on shikimate pathway activity. The experiments were done using a glyphosate-resistant, phyB-deficient Arabidopsis line (gre1) obtained from a T-DNA tagged mutant population. Molecular genetic and physiological analyses confirmed that a knock-out, dominant mutation of PHYB causes the phenotypes. This is consistent with increased glyphosate sensitivity and glyphosate-induced shikimate accumulation in low Red/Far Red light ratio (R:FR), and increase in transcripts of
genes that encode enzymes of the shikimate pathway in high R:FR light. Expression of the shikimate pathway genes exhibit diurnal oscillation and this is altered in the phyB mutant. Furthermore, transcript analyses suggest that this diurnal oscillation is not only dependent on phyB but also due to circadian regulatory mechanisms. Our data offer an explanation of the well-documented observation that glyphosate treatment at various times throughout the day, with their specific composition of light quality and fluence rate, results in different efficiencies of the herbicide.

Keywords: phyB, shikimate pathway, glyphosate resistance

**Poster/Talk #320. Low Energy Stress Response in Arabidopsis thaliana: the SnRK1-bZIP Transcription Factor connection.** (Submission 81)
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Sustaining energy homeostasis is of pivotal importance for living organisms. Limited energy availability is often associated with early events of many biotic and abiotic stress responses. In this situation the plant needs to re-adapt its metabolism to best face the stress. Although Snf1-related protein kinase 1 (SnRK1) and basic leucine Zipper (bZIP) transcription factors (TFs) of the S1 group have already been shown to be important mediators of the metabolic reprogramming in response to low energy availability, conclusive evidence for a functional relation remains elusive. In this study, we present transcriptome-wide expression analyses of inducible loss-of-function approaches targeting all SnRK1s or all group S1 bZIPs, respectively. A substantial set of overlapping genes is reprogrammed upon extended dark treatment, supporting the hypothesis that group S1 bZIPs are regulated by SnRK1s during energy starvation. This reprogramming of gene expression drives the subsequent change from autotrophic to heterotrophic metabolism, where the mitochondrial electron transport chain is assumed to play a major role. In particular, we could show that SnRK1 and bZIP TFs of the S1 group control the expression of Electron-Transfer Flavoprotein: Ubiquinone Oxidoreductase (ETFQO). The reduced expression of ETFQO affects mitochondrial electron transport and leads to the inability of the plant to manage extended dark stress, resulting in an early senescent phenotype. Genetic and pharmacological approaches confirm the importance of an intact mitochondrial electron transport chain in the response to stress that limit the plant's photosynthetic capacity. We could demonstrate that bZIP TFs of the S1 group directly bind to the promoter of ETFQO in a SnRK1-dependent manner, linking starvation-induced SnRK1 activity to regulation of metabolic gene expression. Moreover, we define the mechanisms how SnRK1s are regulating the activity of specific bZIP factors namely, via transcriptional control or phosphorylation-induced dimerization.

In summary, we provide conclusive evidence for a SnRK1/bZIP TFs of the S1 group-dependent energy sensing pathway which is needed to mount a successful adaptive response to Low Energy Stress.

Keywords: SnRK1, bZIP TFs, low energy, mitochondrial electron transport chain, transcriptome

**Poster #321. PEAPOD coordinates cell expansion and proliferation in response to canopy shade in Arabidopsis** (Submission 141)
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When higher plants encounter reduced light quality, due to canopy shading, they adapt by altering their pattern of development. Arabidopsis thaliana seedlings grown under canopy shade conditions responded with a coordinated increase in hypocotyl extension, hypocotyl stomatal density and vascular procambium cell proliferation. In wild type seedlings this coordinated shade avoidance (CSA) response was triggered by a deficit in blue light, but suppressed by high levels of blue light. Seedlings of loss-of-function mutants of the blue light receptors phototropin2 (phot2), cryptochrome 1 (cry1), and cryptochrome 2 (cry2) exhibited a constitutive CSA response when grown in high blue light. A deletion mutant lacking the PEAPOD genes PPD1 and PPD2 (Dppd)1 was also constitutive for increases in hypocotyl elongation, hypocotyl stomatal density and vascular procambium proliferation when grown in daylight, whereas transgenic seedlings over-expressing the PPD1 gene lacked the CSA response. Genetic analyses indicated that the coordinated response utilises a common signalling pathway,
functioning in the order; blue light-PHOT2-CRY1/2-PPD, with PPD acting to limit cell expansion and proliferation in high blue light.

Keywords: Shade avoidance, Signalling pathway, Repressor coordinating growth and development

Poster/Talk #322. MMF1 regulates the photoperiodic control of hypocotyl elongation in Arabidopsis thaliana. (Submission 168)
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Plants alter growth and development to respond to changing environmental conditions, such as day length. This plasticity is the result of the integration of the circadian clock with specific signaling pathways to regulate physiology. However, the components that mediate the photoperiodic control of growth are poorly understood. Using affinity purification and mass spectrometry (AP-MS), we discovered a new protein that directly binds to both clock and light signaling factors, named MASS SPEC IDENTIFIED MODULATING GROWTH FACTOR 1 (MMF1). MMF1 is a conserved, plant-specific, nuclear-localized factor that cycles with a peak at dusk. mmf1 mutant seedlings have elongated hypocotyls in a day-length specific manner, compared to wild type. Conversely, constitutive overexpression of MMF1 shortens hypocotyls, regardless of day length. Affinity purification of MMF1 co-precipitates the circadian-clock associated Evening Complex, red light photoreceptors, and the COP1-SPA complex. Biochemical and molecular assays demonstrate that MMF1 binds directly to EARLY FLOWERING 4 (ELF4) and PHYTOCHROME B. Mutational analysis combined with AP-MS show that phyB is necessary for MMF1 association with clock and light signaling components. Growth assays performed under specific wavelengths suggests that MMF1 regulates red light signaling pathways. Consistently, modulating MMF1 levels alter the expression of genes known to be downstream of the phytochrome signaling pathways, including LONG HYPOCOTYL IN FAR RED 1 (HFR1) and the homeobox transcription factor, ATHB-2 under short day conditions. These results define MMF1 as a new clock and phytochrome-binding factor that participates in growth regulation under photoperiodic conditions. Targeting MMF1 for manipulation could lead to improved growth responses to changing environmental conditions.

Keywords: circadian clock, phytochrome, light signaling, growth

Poster/Talk #323. Interactions between the photoperiodic flowering pathway and the thermosensory pathway in Arabidopsis (Submission 177)
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Flowering time is regulated both by seasonal signals such as photoperiod or winter temperatures, and by ambient environmental cues such as light quality and temperature. In Arabidopsis, long days (LD) and high temperature signals are mediated by different genetic pathways which converge on the transcriptional activation of FLOWERING LOCUS T (FT), a gene encoding a mobile protein that induces flowering in the shoot apical meristem. The B-box transcription factor CONSTANS (CO) is a key component of the photoperiodic pathway which promotes flowering in response to LD by direct activation of FT transcription. On the other hand, thermosensory activation of flowering occurs via two other transcription factors which also affect FT expression: PHYTOCHROME INTERACTING FACTOR 4 (PIF4) and SHORT VEGETATIVE PHASE (SVP). PIF4, a basic helix-loop-helix transcription factor, was shown to be responsible for the thermosensory activation of flowering in short days (SD). PIF4 binds to FT promoter and induces its expression. SVP, a MADS-box transcription factor, is a floral repressor that reduces FT transcription by binding to its promoter. Under warm temperature SVP function is impaired releasing FT repression and accelerating flowering. These observations raise the question of whether the photoperiod pathway might also be active under SD at high temperature and co-operate with the thermosensory pathway in the activation of FT transcription. We found that CO is indeed important for the full activity of the thermosensory flowering pathway under SD. Our results show that co mutant flowers later than WT at 27degC in SD showing a suppression of FT mRNA expression. Moreover svp mutant flowers even earlier in response to warm temperature under SD and this effect is partially suppressed in presence of co mutation. These
we have identified the E3 ubiquitin ligases that are recruited to the PIF3-phyB complex upon light induction and have shown that the multisite phy-induced phosphorylation of PIF3 is required for this recruitment. These ligases then promote concurrent polyubiquitination and degradation of both PIF3 and phyB proteins (1). More recently we have identified the E3 ubiquitin ligases that are recruited to the PIF3-phyB complex upon light induction and have shown that the multisite phy-induced phosphorylation of PIF3 is required for this recruitment. These ligases then promote concurrent polyubiquitination and degradation of both PIF3 and phyB in vivo. These data reveal a linked signal-transmission and attenuation mechanism involving mutually assured destruction (MAD) of the receptor and its immediate signaling partner.


Keywords: Flowering time, Photoperiod pathway, Thermosensory pathway, CONSTANS, PHYTOCHROME INTERACTING FACTOR 4, SHORT VEGETATIVE PHASE

Poster #324. Evaluation of Screening Methods for Salt Tolerance in Soybeans (Submission 247)
Lauretta Ihenatu1, Ken Korth2, Alma Laney2
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Salt toxicity is becoming a major issue in agriculture that adversely affects crop production. Soil salinity increases through salt accumulation caused by repeated irrigation using water with elevated salt content. In soybeans (Glycine max), genotypes that are salt sensitive are termed chloride includers, with Cl- found in all tissues, and genotypes that are more salt tolerant limit Cl- uptake into the leaves are termed chloride excluders. Salinity imposes two major stresses on plants: first, salinity results in a high osmotic pressure in the soil, making it harder for the plant to extract water from the soil; second, salinity causes physiological stress due to the accumulation of Na+ and Cl- in the plant. Evaluation of salt stress under field conditions is difficult because of variable salt levels across the field so greenhouse screening methods have been developed. The objective of this study was to compare four different greenhouse screening methods for evaluating salt tolerance in soybeans. The hypothesis is that there will be no difference among the four screening methods.

Keywords: salt toxicity, soil salinity, soybeans, greenhouse method

Poster/Talk #325. A MAD Mechanism Attenuates Light Signaling in Arabidopsis (Submission 258)
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Following light-induced nuclear translocation, phytochrome (phy) photoreceptors interact with and induce rapid phosphorylation and degradation of bHLH transcription factors, such as PIF3 (Phytochrome-Interacting Factor 3), to regulate gene expression. To define the molecular mechanism of phy-induced PIF3 degradation, we recently performed mass-spectrometric analysis of affinity-purified PIF3 from transgenic seedlings to identify the light-induced phosphorylation sites. Quantitative mass spec analysis indicated that light stimulates phosphorylation of a subset of the Ser/Thr phosphosites identified in PIF3. Using transgenic expression of site-directed mutants of PIF3, we showed that multisite light-induced phosphorylation of PIF3 is necessary for the degradation of both PIF3 and phyB proteins (1). More recently we have identified the E3 ubiquitin ligases that are recruited to the PIF3-phyB complex upon light induction and have shown that the multisite phy-induced phosphorylation of PIF3 is required for this recruitment. These ligases then promote concurrent polyubiquitination and degradation of both PIF3 and phyB in vivo. These data reveal a linked signal-transmission and attenuation mechanism involving mutually assured destruction (MAD) of the receptor and its immediate signaling partner.

Keywords: PIF3(Phytochrome-Interacting Factor), E3 ubiquitin ligase, Light-induced phosphorylation and degradation

Poster #326. F-box and U-box Proteins and their Involvement in the Arabadopsis Circadian Clock (Submission 323)
Ann Feke¹, Kristofof Webb², Steve Kay³, Eric Bennett², Joshua Gendron¹
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In plants, the circadian clock is essential for properly regulating the timing of many growth events, including hypocotyl elongation and flowering time. Traditional approaches for studying clock-dependent gene expression misrepresent a significant portion of the picture, however, as translational and post-translational regulation can lead to a strongly cycling protein from a noncycling transcript, or a steady level of protein from a severely cycling transcript. Standard proteomics approaches are not particularly effective either, as many of the clock proteins are unstable and thus difficult to capture. In this study we demonstrate the preliminary results from a modified proteomics approach, in which the F-box domain is replaced with a HIS-FLAG affinity tag in two Arabadopsis thaliana clock proteins, FKF1 and LKP2. As the F-box domain is that which confers the inherent instability into the protein and its binding partners by being targeted by the SKP-CULLIN ubiquitin ligases, its removal stabilizes the protein while retaining its ability to complex with its protein targets. The added affinity tags can then be used for immunoprecipitation followed by mass spectrometry, allowing for the identification of these proteins’ binding partners. Using this data, the U-box family in plants has been identified as a new avenue for circadian research. Like the F-box proteins, U-box proteins are E3 ubiquitin ligases involved in the degradation of substrate proteins, and thus these same approaches are amenable to their study. Continuing work will identify the role of the U-box family of proteins on the plant circadian clock and their involvement in its regulation and outputs.

Keywords: Circadian Rhythms, Protein Turnover, Proteomics

Poster #327. Investigating the cross-talk between phototropism and shade avoidance in Arabidopsis thaliana (Submission 331)
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Phototropism enables plants to align their growth with the direction of light to optimize light capture in low-light environments. In flowering plants, blue light photoreceptors called phototropins sense the direction of incoming light and trigger the phototropic response. Several studies have shown that the red/far-red light photoreceptors called phytochromes modulate phototropism in etiolated Arabidopsis seedlings; however, their role is secondary to phototropins. Phytochromes, especially phyA is known to enhance hypocotyl phototropic bending. De-etiolated seedlings also display asymmetric growth upon unilateral blue light illumination, however, the regulatory effect of other features of the light environment (e.g. the red/far-red ratio indicative of competition from other plants) on phototropic bending has not been tested in Arabidopsis.

Light-grown seedlings perceive red/far-red light ratio (R/FR) as a cue regarding the quality of light available for photosynthesis. When plants are under foliar shade or near a neighbor, this ratio declines triggering a suite of responses known as the shade-avoidance response (e.g. induction of hypocotyl elongation). In our present study, we have investigated the impact of R/FR on phototropism in Arabidopsis de-etiolated seedlings, or in other words, if phototropic bending in response to blue light gradient is influenced by the surrounding light environment. Our physiological experiments revealed that hypocotyl bending is largely inhibited in high R/FR in a phyB-dependent manner. Genetic analysis suggests that some elements of shade avoidance pathway are involved in regulating phototropism in response to varying R/FR. We hypothesize that seedlings growing under vegetative shade or surrounded by competitors have a physiological advantage to bend towards incoming light to optimize photosynthetic light capture. On the other hand, seedlings in an open field with plenty of light (and a high R/FR ratio) do not need to compete for light and hence do not align towards the incoming light. We are currently investigating the molecular links between phototropism and shade avoidance signaling pathways that co-ordinate the phototropic response with the surrounding environmental conditions.
Keywords: Phototropism, Shade avoidance, Arabidopsis

**Poster #328. DETERMINING CIRCADIAN CONTROL OF GLOBAL PROTEIN DEGRADATION THROUGH DAILY UBQUITYLOME DYNAMICS** (Submission 414)
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The endogenous timekeeping mechanism, known as the circadian clock, is inherent to all forms of eukaryotic life. From the circadian clock, organisms receive a fitness advantage through the ability to coordinate metabolic processes with environmental circumstances. Environmental cues such as light and temperature entrain the circadian clock, allowing for optimized daily phenotype patterns. In Arabidopsis thaliana, genes associated with photosynthesis, hypocotyl elongation, timing of flowering, and resistance to cold all display circadian influence. In understanding the internal driving mechanisms of the circadian clock, much work has been done on the transcriptional and translational details of clock mechanisms. As a result, the plant community now enjoys a relatively robust understanding of the transcriptional network that composes the core of clock function. However, much less is known about the circadian nature of post-translational modification and degradation of protein and how this influences oscillations in protein abundance. The project presented in this poster aims to lessen this dearth of understanding and to produce a deeper understanding of clock controlled protein degradation, which is critical for clock function and connectivity to vital output processes.

By using improved mass spectrometry techniques, and by enrichment of ubiquitylated proteins by immunoprecipitation, we search the entire proteome of A. thaliana for proteins that exhibit a circadian oscillation in their ubiquitylation profiles. We use an antibody that targets the residues of trypsin-digested ubiquitin, which allows us to do analysis at the peptide level, offering increased understanding of the specific nature of the ubiquitylation. We show data supporting the strength and viability of our approach through increased levels of detectable ubiquitin after proteasome inhibition. We also show initial data from 24 hour time courses that show circadian control of ubiquitylated peptides.

Keywords: circadian clock, ubiquitinated proteins, protein degradation

**Poster #329. Investigating the Role of NUCLEAR FACTOR Y Transcription Factors in Light Signaling** (Submission 437)
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Plants perceive light at different wavelengths and integrate the resulting signals to optimize many developmental pathways. Several key transcription factors (TFs) regulating these processes have been identified. We recently demonstrated that one of these key regulators, ELONGATED HYPOCOTYL 5 (HY5), can physically interact with the NUCLEAR FACTOR Y, SUBUNIT C (NF-YC) TFs in yeast two hybrid analyses. This prompted us to examine the roles of NF-YC TFs in light signaling. Because there are 10 NF-YC-encoding genes in Arabidopsis, we examined light-related phenotypes in single, double, and triple nf-yc mutant combinations for several select, highly expressed family members. In addition to reporting the results of these experiments, we will discuss our attempts to more robustly demonstrate putative HY5 by NF-YC physical interactions using fluorescence resonance energy transfer (FRET) analysis in transient tobacco assays. In our initial FRET experiments, we measured fluorescence lifetimes using HY5 and several NF-YC proteins fused to cyan florescent protein (CFP) as donor and yellow florescent protein (YFP) as acceptor. We found some positive results, but were limited by inherent problems in the two chosen FPs (signal intensity, ability to photobleach, etc.). Therefore, we created new Gateway-compatible plant binary vectors harboring fluorescent proteins better optimized for FRET measurements. We will report on our latest findings with these newly developed vectors at the ICAR meeting.

Keywords: Nuclear Factor Y, Light Signaling, Photomorphogenesis, FRET
Poster #330. Identification of suppressors of NUCLEAR FACTOR Y mutants with elongated hypocotyls (Submission 444)
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NUCLEAR FACTOR Y (NF-Y) represents three families of general transcription factors with known roles in development and stress related processes, such as flowering time and ABA signaling. We have recently begun investigating their potential roles in light signaling. We have discovered that various nf-y mutant combinations have elongated hypocotyls when grown in both white and monochromatic light conditions (see also poster presented by Zach Myers). To further explore the roles of NF-Ys in light signaling, we have initiated a forward genetic screen to look for suppressors of the long hypocotyl phenotype. We initially used EMS (ethyl methanesulfonate) mutagenesis on approximately 36,000 Arabidopsis thaliana mutant seeds and have currently screened 100 lots (~50 parental lines/lot) in both the M2 and M3 generations. After retesting putative suppressor lines, we have identified nine independent lines which have suppressed hypocotyl elongation in the nf-y mutant background. These mutant lines are currently undergoing phenotypic analysis for hypocotyl elongation in various light conditions, as well as testing for alleleism groups, confirmation of the recessive or dominant nature of the mutant lines, and verification of the parental genotype. Progress towards these goals will be discussed at the meeting. Upon completion of testing our suppressor lines, we plan to use a next-generation sequencing strategy to identify the causative mutations for the suppressor phenotypes. Ultimately, we hope to identify novel genes in the general light signaling pathways, which can be further explored with molecular, biochemical, and genetic techniques.

Keywords: NUCLEAR FACTOR Y, Light Signaling, Photomorphogenesis, Forward Genetic Screen

Poster #331. The role of the NF-YB histone-like fold in Photoperiodic Floral Induction (Submission 479)
Emily Carpenter¹, Ben Holt¹
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Transcription factors are important proteins that regulate gene expression by binding specific DNA elements in promoters. The heterotrimeric transcription factor NUCLEAR FACTOR Y (NF-Y) recognizes the CCAAT box and is composed of NF-YA, NF-YB, and NF-YC subunits. In higher plants, such as Arabidopsis thaliana, evolutionary expansion of NF-Y subunits have occurred, giving 10 genes encoding each of these three NF-Y subunits. Furthermore, NF-YB and NF-YC proteins respectively have conserved histone-like fold domains (HFDs). NF-YB proteins have known roles in the control of photoperiodic dependent floral induction, with the nf-yb2 nf-yb3 double mutant displaying a strong late flowering phenotype. The other NF-YB members (mutants and/or protein overexpression) showed weaker flowering activity. To understand the role of the different NF-YB proteins, and more particularly their role in the flowering, I performed overexpression assays with each of the 10 NF-YB HFDs to determine if the domain alone is sufficient to activate floral induction. Interestingly, overexpression of each NF-YB HFD, except for NF-YB4, was able to rescue completely or partially the late flowering phenotype of the nf-yb2 nf-yb3 double mutant. This NF-YB functional analysis shows that the HFD can be sufficient to activate flowering, with some different degrees of activity, and that the missing N- and C-terminal domains of NF-YB proteins appear to be evolving as fine-tuning mechanisms for NF-YB function.

Keywords: Photoperiodism, Light, Gene regulation, Transcription factors

Poster #332. Reprogramming Photosynthesis in Arabidopsis thaliana (Submission 495)
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Plants with increased efficiency of photosynthesis will be essential for meeting the future demands of the world population. Even though the photosynthetic apparatus has been under selective pressure for millions of years, the efficiency of photosynthesis in many important crop species typically doesn’t exceed one percent. The aim of this project is to dramatically speed up the evolution of photosynthesis by resetting genome wide transcription patterns and metabolic pathways in the model plant species Arabidopsis thaliana using artificial zinc finger transcription factor (ZF-ATF)-mediated genome interrogation. Due to the nature of their zinc finger modules, ZF-
ATFs are expected to bind at dozens, hundreds or maybe even thousands of different locations in the genome and locally switch on the expression of genes, leading to potentially drastic changes in the transcriptome. Therefore, instead of seeking desired photosynthetic properties in different mutant genotypes, genome interrogation allows us to screen the entire phenotypic space of Arabidopsis for ZF-ATF-induced changes in the efficiency of photosynthesis. We have generated a large library of Arabidopsis mutant lines each harboring a different ZF-ATF encoding gene. This library was subjected to a number of phenotypic screens to find mutants with enhanced photosynthesis. Several mutants with putatively enhanced photosynthesis-related phenotypes have been isolated (e.g. mutants accumulating significantly more biomass than wild type plants; a mutant with significantly higher operating efficiency of Photosystem II; mutants exhibiting a strong increase in tolerance to salinity). In many cases, we have already demonstrated that the respective ZF-ATF-encoding genes harbored by these mutants are the causative agents of enhanced photosynthesis and/or vigor. Using several ‘omics’ techniques, we are now in the process of pinpointing of which genes the expression is up and/or down regulated in these mutants, and therefore, which novel gene expression patterns are involved in improved photosynthesis and growth of Arabidopsis.

Keywords: photosynthesis, zinc finger, artificial, transcription factor

Poster #333. Impact of Manganese stress on Photosystem II in Arabidopsis thaliana. (Submission 504)
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Future sustainable improvement in the productivity of cropping systems depends on novel resource-efficient plant genotypes designed to match site-specific soil and climatic conditions. Deficiency in essential mineral micronutrients such as manganese (Mn), iron, zinc, copper and boron is a significant problem for crop productivity in major parts of the world. Mn is an important microelement in plant nutrition since it is involved in the function of more than 30 enzymes but especially in the oxygen evolving complex (OEC) in photosystem II. Mn is primarily located in the thylakoids where it forms the Mn cluster (Mn4CaO5) which is part of the OEC of Photosystem II. Therefore, Mn deficiency is a serious plant nutritional disorder, occurring mostly on alkaline soils that reduce the bioavailability of this metal. To investigate how the photosystems are affected by Mn stress (both Mn deficiency and Mn excess) a number of selected Arabidopsis thaliana mutants have been grown in a hydroponic system. We have tested the effect of Mn stress on the relative abundance of the proteins of the photosystems and on photosynthetic performance. Induction of Mn deficiency in the Arabidopsis thaliana mutants stn7, stn8, stn7stn8, psbp2 and psbq1psbq2 did not lead to visual symptoms, but all Arabidopsis thaliana mutants tested displayed clear phenotypes when grown under Mn stress conditions. A significant decrease in relative protein abundances of nearly all proteins of the photosystems was observed when subjected to Mn deficiency. The aim of our work is to analyze the general effects of Mn stress in Arabidopsis thaliana. In that context we employ chlorophyll fluorescence measurements in combination with an array of biochemical analyses as well as in depth study of the photosynthetic apparatus.

Keywords: Photosynthesis, nutrient stress, photosystem II, protein

Modeling, Bioinformatics & Systems Biology

Poster #334. Building regulatory networks to predict gene function (Submission 11)
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Transcriptional change is a key element of plant responses to a broad range of stimuli. Understanding how these transcriptional changes are regulated will be crucial to our ability to manipulate plant responses, and the ability to predict regulatory interactions from experimental data will play a key role in this understanding. Network inference algorithms are capable of predicting complex regulatory interactions from time course expression datasets, but their computational tractability and nature often limits the number of genes used in the inference, with the resulting network not being able to reflect the entirety of the interactions featured in the data.
One way to augment such networks is by identifying groups of co-regulated genes. It's a long-standing assumption that co-expression may reflect co-regulation, and using data from multiple conditions and time points can improve the correlation between the two. A recently published algorithm Wigwams can be used to identify groups of genes likely to be co-regulated across subsets of available time series expression data. The algorithm's strength is the ability to discern between statistically significant "dependent" co-expression, indicative of co-regulation, and independent co-expression stemming from the abundance of a particular expression profile. As such, Wigwams is capable of recognising the footprint of shared regulatory interactions between multiple conditions and hence providing groups of potentially co-regulated genes to integrate with existing network models. Six high-resolution time course expression datasets of *A. thaliana* after biotic (*B. cinerea* and *P. syringae* DC3000 infection) and abiotic (long and short day senescence, drought, high light) stimuli were generated. Network inference using the data for transcription factors from these datasets generated network models for each of the stress conditions. Wigwams was then used to mine the entire dataset for gene modules showing dependent co-expression. The potentially co-regulated gene modules were used to expand the network models with non-transcription factor genes, and made it viable to assess the functionality of transcription factors in the network by examining the GO terms of their predicted downstream targets. Several transcription factors, both novel and previously known to be involved in the defence response, were predicted to be involved in hormone signalling in *B. cinerea* and *P. syringae* infection.

Keywords: Gene regulation, Network model, Data mining, Gene function prediction

**Poster #335. Identifying Chloroplast Thiamine/TPP transporter(s) (Submission 67)**
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Abstract: Thiamine pyrophosphate (TPP) serves as a cofactor in universal metabolic pathways including glycolysis, the pentose phosphate pathway, and the tricarboxylic acid cycle, and is essential for the proper functioning of all organisms. Recently, several steps of the plant thiamine biosynthetic pathway have been characterized, and a mechanism of feedback regulation for the thiamine biosynthesis via a riboswitch mechanism has been unravelled. In plants, thiamine is made in the chloroplast and is then transferred to the cytosol to form the active form of thiamine, thiamine pyrophosphate (TPP) by thiamin diphosphokinase (TPK) which appears to be exclusively cytosolic. The mitochondria and chloroplast must import TPP from the cytosol because both chloroplast and mitochondria contain TPP-dependent enzymes. In Arabidopsis, two members of the mitochondrial carrier family (MCF), AtTpc1 and AtTpc2, export TPP to the mitochondria, but the chloroplast TPP carrier is still unknown. This study aims to identify thiamine and TPP chloroplast transporter(s). Phylogenetic analysis has shown that there were two chloroplast thiamine transporters (CTT 1 and 2) have similarity to TPP known transporters and have homology in C-terminal regions. Using a reporter gene system, both transporters (CTT1 and 2) target to chloroplast. Using 3D structural predictions of known TPP transporters we have shown that the putative chloroplast transporters CTT 1 and 2 transporter have a similar structure. To test this hypothesis we are using a combination of insertion mutant analysis and transient expression studies.

Keywords: Thiamine, Thiamin diphosphate carrier, Chloroplast, Membrane transport

**Poster/Talk #336. Modeling stem cell networks to identify key regulators of plant growth (Submission 122)**
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Elucidating the mechanisms by which a stem cell divides to reproduce itself and a daughter cell with a different fate is a key challenge for understanding the development in multicellular organisms. Identifying the critical features of stem cell regulatory networks is a necessary step towards controlling plant development. In the Arabidopsis root, key pathways that regulate stem cell development have been uncovered. However, past studies have not fully addressed the dynamics of these regulators, nor the regulatory interactions between their network components. Accurate quantitative characterization of the key components of these networks is essential to generate models that capture the behavior of the system. Our goal is to experimentally identify the essential features of these regulatory networks and develop accurate mathematical models that describe stem cell network
dynamics. To experimentally quantify molecular dynamics, we are using fluorescence correlation spectroscopy techniques, which enable in vivo quantification of specific parameters such as protein mobility, concentration, and associations. Transcription factors have been shown to play key roles in regulating the processes of stem cell development. The ability to obtain quantitative information about the dynamics of intercellular movement and interaction among different transcription factors is critical for a systems-level understanding of stem cell maintenance. Parameter values for key transcription factors involved in our stem cell network have been obtained. These experimentally determined parameters are used to generate a set of equations to model the dynamics of this network in a spatial context. The integration of imaging tools with the modeling of the regulatory networks offers the unique advantage of monitoring the function of biological circuits over time at cellular resolution.

Keywords: plant stem cells, imaging, modeling, integration across scales, transcriptional modules

Talk #337. Knowledge-based bioinformatic analyses of microarrays predict that epigenetic regulator AS1-AS2 controls cell division through ETTIN in leaf adaxial-abaxial patterning (Submission 136)

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It is necessary to use algorithms to analyze gene expression data from DNA microarrays, such as machine learning and pattern recognition. Previously, we developed a filtering method based on the projective adaptive resonance theory (PART), a high dimensional clustering algorithm for marker gene selection of cancer diagnosis. We also developed a knowledge-based fuzzy ART (KB-FuzzyART) to find clues for identifying gene networks in plant development. Although KB-FuzzyART requires any gene lists, this clustering method is more accurate and interpretable than conventional clustering methods. We applied KB-FuzzyART to study gene regulatory mechanisms in leaf development. Leaf primordia form around the shoot apical meristem (SAM), which consists of indeterminate stem cells. Upon initiation of leaf development, adaxial-abaxial patterning is crucial for lateral expansion, via cellular proliferation, and the formation of flat symmetric leaves. Despite the crucial role of the ASYMMETRIC LEAVES1 (AS1)-AS2 nuclear-protein complex in early leaf partitioning into abaxial and adaxial domains, information on mechanisms controlling their downstream genes has remained elusive. We have systematically analyzed expression array by KB-FuzzyART and ChIP-chip and identified an AS1-AS2 target gene, ETTIN (ETT)/AUXIN RESPONSE FACTOR3 (ARF3), which encodes an abaxial factor. While the AS1-AS2 complex represses ETT by direct binding to the ETT promoter (TGS), it also indirectly activates miR390- and tasiR-ARF-dependent post-transcriptional gene silencing (PTGS) to negatively regulate both ETT and ARF4 activities. We have further analyzed expression arrays of double mutants (as2 and its modifier genes) by KB-FuzzyART and knowledge-based enrichment analysis to identify genes downstream of ETT. Bioinformatic analyses and subsequent molecular genetic analysis have showed that expression of Kip-related protein (KRP) (for inhibitors of cyclin-dependent protein kinases) and Isopentenyltransferase (IPT) (for biosynthesis of cytokinin) genes were controlled by AS1-AS2 through ETT and ARF4 functions. Furthermore our recent genetic analysis showed that the krp5 mutation suppressed abnormality of adaxial-abaxial polarity in the as2 mutant. These results suggest that the AS1-AS2 and ETT-KRP5 pathway plays a critical role in controlling the cell division cycle around SAM to establishment of leaf polarity development in Arabidopsis thaliana.

Keywords: knowledge-based bioinformatics, clustering, microarray, gene expression, leaf polarity, ETTIN/AUXIN RESPONSE FACTOR3 (ETT/ARF3), KIP-RELATED PROTEINS (KRP5), ASYMMETRIC LEAVES1 (AS1), ASYMMETRIC LEAVES2 (AS2)

Poster #338. NetExCorr: A Pipeline to Rank Gene Prevalence in Tissue-Specific Responses to Stresses within Arabidopsis in-vitro Meta-Network. (Submission 225)

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Part of the challenge to increase crop yield while decreasing plants’ susceptibility to various stresses, is to understand plant tissue-specific responses to to stress combination, as it occurs in natural environment.
conditions. miRNA and transcription factors are key parts of this understanding, as they participate in the development and stress cross-talks. In order to find and to rank putative regulators at the intercept between developmental and stress processes, we generated a computational pipeline based on multiple-criteria decision-making algorithm called NetExCorr. With the objective of ranking genes in a selected condition (tissue and/or stress), NetExCorr uses network proprieties and overlay with global gene expression analysis. Hence, NetExCorr is based on 5 weighted parameters: three in-vitro network topology statistics (centralities and transitivity), condition-specific expressions and significant co-expressions present within an in-vitro network. Since most global expression data do not include reliable miRNA expressions, NetExCorr also includes a method measuring the expression effect of miRNAs. To reach this objective, the pipeline includes the MIR software, which takes in account miRNA-target expressions and miRNA/mRNA binding affinities to estimate the expression effect of miRNAs with a GSEA-like method. To adapt this MIR tools to plants, we generated an algorithm enabling an estimation of miRNA-mRNA binding affinities in plants. Preliminary validations of this algorithm showed a 78 to 93% correlations between binding affinity estimations and the in vivo transcriptional inhibitions of targets from miR165. As a model for NetExCorr, we have merged an Arabidopsis in-vitro meta-network that regroups interaction maps from the literature containing transcriptional regulation (miRNA based network and AtRegNet), post-transcriptional regulation (miRNA/mRNA interactions), protein regulation (protein-protein interactions) as well as post-translational regulations (phosphorylations). This meta-network contains 35K interactions for 11K genes. After the validation of NetExCorr using root spatio-temporal data, we applied this pipeline to rank genes in the Arabidopsis meta-network for drought in combination with heat, cold or salinity in roots. We are currently in the validation process for the putative candidates found.

Keywords: Gene prioritization, miRNAs, In-vitro network, Tissue-specific expression, Co-expression, Stress and Development

**Poster #339. TAIR: A Sustainable Community Database for International Arabidopsis Research** (Submission 229)
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Started as a NSF-funded project in 1999, TAIR has served as the primary source and the main portal of curated Arabidopsis data, with worldwide usage of 66,000 visitors per month and 2.4 million visits per year. Over the past year TAIR has transitioned to a new funding model supported by institutional and individual subscriptions. This model provides a sustainable path for TAIR to continue to serve the increasing needs for high quality curated data from the international Arabidopsis and plant research community. We will present the following: 1) the rationale for TAIR to move to a more sustainable funding model supported by subscriptions (non-profit); 2) an update on the progress we've made so far in developing this new funding mechanism; 3) the steps we've taken to maximize the number of researchers with access to TAIR data by offering a variety of subscription options (national, institutional, individual and complimentary access for teaching); 4) vision for the future development of TAIR.

Website: [http://www.arabidopsis.org/](http://www.arabidopsis.org/)

Keywords: Arabidopsis, database, bioinformatics, sustainability

**Poster #340. Arabidopsis Information Portal (AIP): a user's perspective** (Submission 246)
Chris Town¹, Jason Miller¹, Matt Vaughn², Gos Micklem³, Eva Huala⁴, Benjamin Rosen¹, Bob Muller⁴, Erik Ferlanti¹, Maria Kim¹, Svetlana Karamycheva¹, Vivek Krishnakumar¹, Sergio Contrino³, Julie Sullivan³, Matt Hanlon⁵, Rion Dooley⁶, Steve Mock²
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The Arabidopsis Information Portal (AIP; [http://www.araport.org](http://www.araport.org)) is a new on-line resource for plant biology research. AIP was funded by NSF and BBSRC starting in 2013 to provide users with a single interface through which to access a wide range of Arabidopsis information - a "one-stop shop" - using state-of-the-art web technologies. As part of this mandate, AIP inherited from TAIR the responsibility for providing free access to up-to-date structural and functional annotation for the Col-0 genome. AIP currently hosts most of the TAIR10 data.
content and integrates it with data from UniProt, PubMed, BAR, EPIC-CoGe, and other sources. Later this year, AIP will release the AIP11 annotation update containing new splice variants computed with tissue-specific transcript assemblies of RNA seq data. Future releases will incorporate community curation facilitated by AIP. AIP users can query and view the data with powerful GMOD software including InterMine, JBrowse and GBrowse. Users can not only save results but manipulate them, visualize them, and expose them as web services. Users can read news, link out to stock centers, leverage iPlant resources, and interact with other users. Designed for sustainability, the AIP strategy exploits existing scientific computing infrastructure, adopts a practical mix of data integration technologies, and encourages its user community to contribute suggestions, data sets, web services, and software.

Keywords: Informatics, Genome annotation, Data integration, Araport

**Poster #341. Arabidopsis Information Portal (AIP): a contributor's perspective** (Submission 256)
Matt Vaughn1, Jason Miller2, Chris Town2, Gos Micklem3, Erik Ferlanti4, Vivek Krishnakumar4, Svetlana Karamycheva4, Maria Kim4, Ben Rosen4, Sergio Contrino3, Julie Sullivan3, Matt Hanlon1, Rion Dooley1, Steve Mock1
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The Arabidopsis Information Portal (https://www.araport.org) is an open-access online community resource for Arabidopsis research. AIP enables biologists to navigate from the Arabidopsis thaliana col-0 reference genome sequence to its associated annotation including gene structure, gene expression, protein function, and interaction networks. AIP is designed to grow with the Arabidopsis community by providing an extensible framework for incorporation of new data sources and creation of user interfaces to consume them. AIP relies on a data warehouse (InterMine) to provide a searchable index of sequence-associated data. AIP relies on data federation to deliver in-depth information at run time. To support data federation, AIP uses innovative middleware based on the iPlant Agave platform to support enrollment, discovery, and access to community-developed web services within a unified framework. AIP incorporates and integrates software from GMOD including InterMine, JBrowse, GBrowse, WebApollo, Tripal, and Chado, and other open-source technologies. The AIP project itself is fully open source, maintaining an active GitHub repository and virtual organization. AIP hosts tutorials, workshops, and hackathons to help the community enrich and exploit this resource.

Keywords: Bioinformatics, Web services, Araport, AIP, Data integration, TAIR

**Poster/Talk #342. A proteomic strategy for global analysis of protein complex composition and localization in Arabidopsis leaves** (Submission 264)
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Protein complexes are fundamentally important for diverse types of cellular regulation. For example, oligomerization of glycolytic enzymes at the mitochondrial surface can shuttle metabolites into the mitochondria. Sequentially assembled protein complexes precisely execute mechanical tasks like chromosome segregation, vesicle budding, and long distance intracellular transport. Protein complexes can also relay information. They change localization, conformation, and/or assembly status to convert input signals, often from multiple sources, into a coordinated cellular response. Experimental data concerning protein complexes therefore, provide important clues about cellular pathways and their regulation. Currently, affinity purification-mass spectrometry is a commonly used approach to discover endogenous protein complexes. However, this method is not suitable for organisms that are not easily transformed, and protein-tag might affect the expression level of endogenous proteins. There is a strong need to develop additional methods for global analysis of protein complexes in cells. Here we describe a new approach that capitalizes on the ability of mass spectrometry to quantify thousands of proteins within a single run. Our method begins with intact leaves, which is used as the source of a crude cytosolic and organelle fractions. Soluble protein complexes were separated using multiple chromatographic techniques, and the fractions were analyzed using label-free quantitative mass spectrometry. The analysis
generated elution profiles for thousands of proteins, which can be used to determine the size and composition of protein complexes based on clustering analysis. We used this approach to discover hundreds of proteins that appeared to exist as components of stable protein complexes in the cytosol. The reliability of the method was validated by western blotting, comparisons with the published size exclusion chromatography data, and the masses of known protein complexes. The reproducibility of the method was analyzed in biological replicates. In principle, this method is suitable for any organism, cell type, or subcellular compartment without the need for any genetic manipulation. We will provide a summary of our progress in using this approach to detect protein complexes, determine their localization, and predict their composition as a function of metabolic stress.

Keywords: Arabidopsis, Protein complexes, Chromatography, Mass spectrometry, Metabolic stress

Poster/Talk #343. An organ boundary-enriched gene regulatory network uncovers regulatory hierarchies underlying axillary meristem initiation (Submission 268)
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Development is controlled by gene regulatory networks (GRNs), which involve cell type-specific genome expression and interactions between transcription factors (TFs) and regulatory promoter regions as the primary level of control. Plant organ boundaries separate lateral organs from the meristem, which contains stem cells, and harbor axillary meristems (AMs). AMs, as new stem cell niches, make the whole shoot a ramifying system. Albeit important for plant development, our knowledge on organ boundary and AM formation remains incomplete. Here, we generated a cellular resolution genome expression map for Arabidopsis thaliana organ boundary cells, and obtained a genome-wide protein-DNA interaction map focusing on genes affecting boundary and AM formation. The delineated GRN uncovers transcriptional signatures, predicts new cellular functions, and identifies promoter hub regions that are bound by a large number of TFs. Importantly, further experimental studies determined regulatory consequence of many TFs for their targets, including new regulators and regulatory relationships of AM initiation. This study provides a striking example where a systems biology approach speeds our understanding of a developmental process.

Keywords: axillary meristem, gene regulatory network, organ boundary

Poster #344. The growth of the tissue predicts the growth of individual cells in Arabidopsis sepals (Submission 346)
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Organogenesis is robust; mature organs have characteristic sizes and shapes that are nearly invariant across individuals. Yet, the growth and division of their individual cells is variable in space and time. A key challenge is to determine how regularity in organ size emerges from the stochastic behaviors of individual cells. To address this question, we have analyzed how the growth of individual cells in the Arabidopsis sepal relates to the growth of the overall organ. Arabidopsis floral organs are particularly amenable for studying organogenesis because a large number of identical organs are produced on the same plant and their growth is fairly insensitive to environmental variations. We imaged sepals on living plants expressing a fluorescent plasma membrane marker every 6 hours for at least 66 hours. To analyze their growth, we have developed and used a new tool for tracking cell lineages in the MorphoGraphX image analysis software.

We find that both the growth of the cell area and length of individual cell walls fit sigmoidal curves. Additionally, we observe a linear relation between the maximal growth rate within the sigmoidal curve and the size of cells and walls. The growth of individual cells appears somewhat variable, differing between neighbors, although spatial trends from the top to the bottom of the sepal are present. Most of the variability observed occurs in the timing and the amount of growth, where larger cells are associated with longer growth times. Furthermore, we find that the cell's growth stages do not obviously correlate with the area or orientation within the sepal or its cell divisions.
To determine how the growth of cells relates to the tissue, we first approximate a continuous low-order deformation gradient that describes the overall growth of each sepal. Based on this deformation gradient we predict the growth of each cell or cell lineage in the sepal over time. We find that the predicted growth of the cell or cell lineage based on the tissue matches well with the actual growth of the cell or cell lineage measured in the data. We suggest that the variability we observe in growth at the cellular level is a consequence of the tissue growth subject to spatial variations in the timings of growth.

Keywords: sepal, growth, image processing, organogenesis, MorphoGraphX, cell lineage

Poster/Talk #345. Gramene: A Resource for Comparative Plant Genomics and Bioinformatics (Submission 429)

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Gramene (www.gramene.org) is acurated resource for comparative functional genomics in model and reference plant species. Its strength derives from the application of phylogenetics and integration of genome annotation and functional data using ontologies. Produced in collaboration with the Ensembl Plants, the current release features 33 complete reference genomes of plants, including the reference Arabidopsis thaliana, A. lyrata and Brassica rapa. For each reference genome, we incorporate community annotation from primary sources followed by an enriched functional annotation by InterProScan and classification using controlled vocabularies, Gene/Plant Ontology (GO/PO). Evolutionary histories are provided by Compara phylogenetic gene trees and complemented by analyses of whole-genome alignments. In recent years Gramene has positioned itself as a resource for genome variation data. The current release includes ~14 million Arabidopsis SNPs and indels identified in hundreds of ecotypes by the Arabidopsis 1001 Genomes Project and several national and international projects. Gramene also produces and hosts the Plant Reactome and Ptools-based metabolic pathway databases and visualization tools. Plant Reactome (http://plantreactome.gramene.org), is a platform for the comparative analysis of plant metabolic and regulatory networks, featuring at present curated metabolic and regulated pathways of rice and Arabidopsis and orthology based projections to maize. In collaboration with the Gene Expression ATLAS project (http://www.ebi.ac.uk/gxa) we have analyzed numerous publicly available Arabidopsis and rice gene expression datasets that are now available for query and browsing on the ATLAS database website. Gramene is supported by an NSF award #IOS-1127112 and works closely with its partners, namely the EBI-EMBL, the Reactome Knowledgebase, and the American Society of Plant Biologists (ASPB).

Keywords: Plant genomics, Plant Pathways, Bioinformatics, Arabidopsis genomes, Arabidopsis gene homologs, Genome alignments, SNP, Arabidopsis variation, genetic variation, networks, pathways, gene expression, genome, rice, maize, pathway database, database

Poster #346. Periodic gene expression precedes lateral root formation (Submission 448)

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Periodic gene expression in the oscillation zone (OZ, Moreno-Risueno et al., 2010) of Arabisopsis roots marks the pre-branch sites (PBS), which define the cells competent to develop into lateral root primordia. To further define the mechanism underlying this oscillation, we are taking a three-fold approach. First, we aim to identify the genes and network that regulate this periodic behavior. To do this, we performed live imaging of DR5::LUCIFERASE (marks the OZ) transgenic Arabidopsis seedlings and micro-dissected five regions: the OZ at a time when expression is in its highest point ("in-phase"), the first PBS and the flanking regions. These sections were used to prepare RNA-seq libraries to identify differentially expressed genes by whole transcriptome sequencing. In parallel, we generated a mutagenized DR5::LUCIFERASE population and will identify mutants that affect oscillation. The RNA-seq and mutagensis-screen results will be compared to identify genes likely to regulate the
root clock. We will further investigate the role of these genes by molecular, genetic and biochemical methods. Secondly, we are testing the hypothesis that the oscillating gene network requires tissue context and communication between cells; alternatively, the periodic expression is cell-autonomous and communication is necessary only for synchronization of periodic gene expression. We developed a protocol that allows us to visualize DR5::LUCIFERASE protoplasts for long periods (~7-10 days) in order to test this hypothesis. We will image DR5::LUCIFERASE as well as other protoplast lines in which LUCIFERASE is driven by constitutive (e.g., UBQ10) or oscillating (e.g., the circadian CCA1) promoters and compare the expression patterns between these populations. We will manipulate cellular interaction, e.g., by blocking plasmodesmata channels in planta to understand the importance of inter-cellular signaling within the OZ. Lastly, we are examining the evolutionary prevalence of the root clock; we will assay DR5::LUCIFERASE expression in transgenic rice, tomato and c-fern (Ceratopteris richardii) to determine whether the root clock is a broad phenomenon in the plant kingdom.

Together, this study will facilitate our understanding of the root clock and more broadly, the mechanisms underlying oscillatory behavior during plant development.

Keywords: oscillation, lateral root, RNA-seq

**Poster #347. Modeling gene regulatory networks that control Arabidopsis stem cell identity** (Submission 463)

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d, Adam Fisher
d, Natalie Clark
d, Morjan Rahhal
d, Cranos Williams
d, Ross Sozzani
d

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Understanding stem cell behavior is key to improving growth and development of plants. However, many of the mechanisms that regulate stem cell behavior, such as those underlying stem cell identity and differentiation, are still unknown. Identifying and mathematically characterizing new gene regulatory networks controlling stem cells in Arabidopsis is a step towards elucidating these mechanisms.

To unravel these networks, we are characterizing the transcriptional profile of each of the root stem cell populations during development. We have used clustering-based bioinformatics methods to identify genes involved in stem cell identity and maintenance. For this, we relied on the rationale that enrichment in gene expression is a proxy for gene function. We have identified 67 genes, each of which is enriched in all of the stem cell populations but not expressed in non-stem cells. This set of genes contains a total of 6 transcription factors (TFs) for which we are building Bayesian networks to model their gene regulatory networks.

Our research may reveal if a master regulator controls the stem cell niche and its maintenance. We are doing this by identifying causal relationships among these TFs and their downstream targets and leveraging the network topology to assess the importance of genes. The identified TF-gene relationships will be used to infer mechanistic models (i.e. ODEs with Hill functions) for the key players involved in stem cell regulation. System analysis of the generated model will determine the emergent behaviors underlying stem cell maintenance. Our approach is the first step toward a predictive mechanistic model of how stem cell networks function and are regulated, which is a fundamental tool for engineering and manipulating stem cells.

Keywords: Systems biology, Plant development, Stem cells

**Poster #348. Application of Petri nets to the analysis of auxin-driven plant morphogenesis** (Submission 466)

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d

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One of the most fascinating aspects of plant morphogenesis is the ubiquitous role of the growth regulator auxin. Over the last decade, the analysis of auxin-driven morphogenesis based on experimental data has been enhanced with the wide use of computational models. These models have confirmed the plausibility of several previously proposed concepts and provided explanations to apparent contradictions (e.g., the experimentally reported high concentration of auxin in veins, contrary to the original interpretation of the canalization hypothesis). The use of models has also sharpened the central question of auxin-based patterning: what mechanisms govern the feedback between auxin concentrations, its fluxes, and PIN polarization in cells.
Several models addressing this question have been introduced in the literature, but their interpretation and comparisons are not straightforward. To facilitate the analysis, we propose to visualize the hypothetical mechanisms of PIN1 polarization and auxin transport using Petri nets, an intuitive graphical representation of distributed systems with applications to biochemical networks. Our analysis begins with the up-the-gradient model of PIN1 polarization introduced by Jonsson et al. (2006) and Smith et al. (2006) to explain phyllotactic patterning. Using Petri nets, we show how subsequent models that suggest a mechanistic explanation of the up-the-gradient polarization can be related to the original model and to each other.

We extend our analysis to with-the-flux models, introduced by Sachs (1969) to explain vascular patterning. Using Petri nets, we compare previously proposed models that aim at explaining the mechanisms through which with-the-flux polarization may be induced. We also propose a new, relatively simple model, in which PIN polarization can shift from up-the-gradient to with-the-flux mode depending only on the rates of reactions.

In conclusion, Petri nets provide an effective method for elucidating the biochemical mechanisms that may govern the feedback between auxin concentrations, its fluxes, and PIN polarization. Petri nets also facilitate the construction of new models that are automatically consistent with the law of mass action and thus meet the fundamental requirement of biochemical plausibility.

Keywords: PIN polarization, Petri nets, biochemical mechanisms

Natural Variation, Ecology, Evolution

Poster/Talk #349. Genetic basis of natural variation in heat-stress response in Arabidopsis thaliana: A genome wide association study. (Submission 28)
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Fruit setting in tomato and other plants is very sensitive to deviations from optimal temperatures. A single hot day may already result in a reduction in yield. Breeding programs for heat tolerance can be improved if more is known about genes playing a role in heat response and tolerance. Our research aim is to elucidate genes regulating this observed reduction in fertility. We investigated the natural variation for the response to heat in a population of 350 diverse accessions of the model species Arabidopsis thaliana. Plants were exposed to a single day of 350°C, one week after the start of flowering. Fertility was evaluated by measuring the length of all siliques along the main stem three weeks after the treatment. Siliques length is a good measure for fertility as it is highly correlated with seed set. Strong natural variation was observed, ranging from no effect up to 70% reduction in seed yield. The developmental stage of the flower at the time of the heat stress had a large influence on the response. Two sensitive stages were observed. The first shortly after fertilization, which means that early stages of embryogenesis are more sensitive than later ones. The second sensitive stage was observed for flowers that had opened three or four days after the treatment. At this stage male and female meiosis take place in the flowers. Genome Wide Association (GWA) mapping resulted in the detection of 74 QTLs. In silico analyses of the underlying genes resulted in the selection of 50 candidate gene. Among them are genes specifically expressed in male gametophytes and some genes known to be involved in flowering time regulation. Validation of candidate genes is currently undertaken through knock-out and over-expression studies and complementation tests. Subsequent translation of findings towards tomato will enable marker assisted breeding for varieties with a high performance at a wider range of temperatures.

Keywords: natural variation, Genome Wide Association Studies, GWAS, Heat stress, Reproductive stage, Fertility

Poster #350. Natural variation in temperature perception and response in Arabidopsis thaliana (Submission 73)
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Temperature affects almost all aspects of plant development. The molecular processes underlying temperature perception and response have been of interest in our group. We have identified two key factors required for plant growth at higher temperature through a combination of mutant analysis of natural variation. Our results reveal upstream processes in temperature signalling. Higher temperatures reveal cryptic phenotypes and the analysis of these phenotypes have resulted in the identification of an important factor required for plant growth specifically at higher temperatures. I will describe our recent work on these specific factors and our global analysis of the genetic architecture of temperature response in Arabidopsis thaliana.

Keywords: Natural variation, Temperature perception, QTL cloning

**Poster #351. Single-locus hybrid necrosis caused by common, co-occurring ACD6 alleles.** (Submission 86)
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Resistance to pathogens requires fine-tuning of a complex and constantly evolving immune system. A fundamental feature of this defense system is the ability to distinguish self and non-self. In plants, self-recognition involving immune regulators can sometimes present itself in hybrids, either inter- or intra-specific, leading to an autoimmune response; affected plants grow less well and suffer from spontaneous cell death, but are at the same time more resistant to pathogens. Known instances of hybrid necrosis generally involve epistatic interaction between unlinked loci, and are often caused by relatively rare and/or geographically distant alleles. We identified the first case of single-locus hybrid necrosis, in which interactions between two classes of natural alleles of the ACCELERATED CELL DEATH 6 (ACD6) gene lead to the activation of the Arabidopsis thaliana immune system. One of these allelic classes has recently evolved by partial resurrection of a pseudogene, and each class includes multiple functional variants. The two classes are particularly common in the Northeast of Spain, suggesting a role in local adaptation. In this area, these alleles co-exist in natural populations, and hybrid individuals carrying combinations of the two ACD6 allelic classes can be found as well. The extensive functional variation among ACD6 alleles points to a central role of this locus in fine-tuning pathogen defenses in natural populations.

Keywords: Hybrid necrosis, Immune system, ACCELERATED CELL DEATH 6 (ACD6), pseudogene resurrection

**Poster/Talk #352. An evolutionary perspective on plant epidermal patterning** (Submission 115)
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Mechanisms driving phenotypic changes can be studied through evolutionary developmental biology. Research on plant epidermal patterning has been focused on Arabidopsis thaliana (A. thaliana) and is controlled by the WD40 protein TRANSPARENT TESTA GLABRA1 (TTG1). TTG1 regulates five traits relevant for the adaptation of plants to environmental changes including the production of proanthocyanidin, anthocyanidin, seed coat mucilage, trichomes and root hairs. Due to their adaptive value for the plant, these 5 traits are likely to be variable on the one hand, but also interdependent as they are controlled by the same regulatory genes. Presently, in plant-developmental biology, evolutionary comparisons comprise a few well-characterized model systems separated by large evolutionary distances leading to a rather descriptive view, than having functional depth. The analysis of different Brassicaceae species suggests that the function of TTG1 is conserved within the family. In order to obtain a functional evolutionary comparison of a developmental process like epidermal patterning, it needs to be studied in related species of the same taxon. To this end we have established Arabis alpina (A. alpina), a sister Brassicaceae as a genetic model system to study the evolution of TTG1-dependent gene regulatory network at a functional level. We searched for EMS mutants affecting trichome and root hair patterning in A. alpina to understand its genetic basis. This revealed numerous patterning mutants reminiscent of the ones in A. thaliana.
but also new mutant phenotypes were discovered. In parallel we identified these patterning genes by sequence similarity and synteny, laying the foundations for a comprehensive study of evolution of a whole gene regulatory network. A comparison of wild type and two Aattg1 alleles; discovered in the EMS screens; revealed that AaTTG1 is involved in the regulation of all five traits. A detailed analysis of the five traits showed striking phenotypic differences between A. alpina and A. thaliana such that trichome formation occurs also at later stages of leaf development and that root hairs form at non-root hair positions. The evolutionary conservation paired with striking phenotypic differences of TTG1-dependent traits makes A. alpina an attractive evodevo model. We envision this would cast a new light on the evolution of plant epidermal patterning at a functional level.

Keywords: Arabis alpina, TTG1, plant epidermal patterning, trichomes, root hairs, pro-anthocyanidin, anthocyanidin, seed coat mucilage

Poster/Talk #353. Recovering a triplet expansion in the wild: Repeat expansion and phenotypic variation in Irish populations of Arabidopsis thaliana. (Submission 139)
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The Bur-0 accession of Arabidopsis thaliana contains an intronic (GAA)n expansion in the ISOPROPYL MALATE ISOMERASE LARGE SUBUNIT1 (ILL1) gene resulting in the cryptic "irregularly impaired leaf (iil)" phenotype, only evident at higher temperatures. Analysis of (GAA)n tract length at the IIL1 locus across 95 globally distributed A. thaliana strains revealed a normal distribution of 0-38 repeats, with the exception of the Bur-0 accession (>400 repeats). The repeat expansion is unique only to the Bur-0 strain, which is originally collected from the Burren region in Ireland. The Burren region is renowned for its unusual assemblage of rare plant species owing to its unique glacio-karst limestone ecology. In an effort to ask whether this repeat expansion still persists in nature and whether it is more frequent in Ireland compared with global populations, a population genetic study was undertaken across Ireland with >500 samples collected from 200 separate locations over a 2-year period. Considerable variation in (GAA)n repeat tracts was observed with the exceedingly rare dramatically expanded allele uncovered in 8 subpopulations. The high frequency of this TRE in Ireland potentially indicates the conditions either favour or at least do not warrant removal of the expansion. Genetic diversity and phylogeny was characterized through DArT sequencing of 150 accessions from 60 subpopulations across Ireland generating 11,242 SNPs. Population physiology and diversity was also analysed across >20 phenotypic parameters and association mapping performed using MLM with TASSEL software. Several positive associations were uncovered including associations with the phenotype of (GAA)n repeat length itself. Identifying genetic regions associated with the maintenance of tandem repeat length allows further investigation of identified candidate genes for their potential functional roles in repeat length variability.

Keywords: Arabidopsis thaliana, triplet repeat expansion, association mapping, Burren

Poster #354. Protein subcellular relocalization after gene and genome duplication during the evolution of the Brassicaceae (Submission 140)
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Gene duplications during eukaryotic evolution, by successive rounds of polyploidy and by smaller scale duplications, have provided an enormous reservoir of new genes for the evolution of new functions. Preservation of many duplicated genes can be ascribed to changes in sequences, expression patterns, and functions. Protein subcellular relocalization (protein targeting to a new location within the cell) is another way that duplicated genes can diverge. We studied subcellular relocalization of gene pairs duplicated during the evolution of the Brassicaceae, including many gene pairs from the alpha whole genome duplication. We analyzed experimental localization data from green fluorescent protein experiments for 128 duplicate pairs in Arabidopsis thaliana, revealing 19 pairs with subcellular relocalization. Many more of the duplicate pairs with relocalization than with the same localization showed an accelerated rate of amino acid sequence evolution in one duplicate, and one gene showed evidence for positive selection. We used gene family analysis with several pairs to determine which gene shows relocalization. We identified sequence mutations resulting in neolocalization of three duplicated gene
products through comparative analysis, including as few as two amino acid mutations at the N- or C-terminus and mutations resulting in loss of an N-terminal localization signal. We show that four cases of relocation have undergone regulatory neofunctionalization including two in pollen. This study provides insights into sequence evolution, regulatory neofunctionalization, and types of sequence mutations that cause subcellular relocation of evolutionarily recent gene duplicates in flowering plants.

Keywords: molecular evolution, duplicated genes, whole genome duplication, subcellular relocalization, neofunctionalization

**Poster/Talk #355. Natural variation of a gene network regulating trichome patterning** (Submission 147)  
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In Arabidopsis thaliana (A. thaliana), trichome patterning, root hair patterning, seed coat mucilage formation, anthocyanin and proanthocyanin biosynthesis are regulated by an evolutionary conserved gene network governed by TTG1. This is done with different combinations of bHLH and MYB genes for each trait. These traits are likely to be naturally variable as it has been shown that they are involved in protection against pathogens, protection against UV, germination, etc. An increasing number of A. thaliana accessions, as well as their genome sequences, are available and their variation in traits such as trichome patterning can be used as a tool to unravel the mechanisms driving developmental processes. In this work, we focused on the first set of 80 accessions for which the sequences were released in 2011. To capture the different classes as well as the development of trichomes, we analyzed the third true leaf at a stage when it is approximately 100um long. We developed TrichEratops (a software that allows a semi-automated 3D leaf reconstruction) to screen the accessions. In the analyzed set of accessions, large variations in different aspects of trichome patterning were found. We combined Genome Wide Association Mapping and candidate gene approaches to study the natural variation. Using the known epidermal-patterning mutants as a reference we apply correlation analysis, using specific trichome patterning parameters and sequence analysis to identify accessions having variation in this pathway. As the full genome of more than 800 accessions is now available, we compute Linkage Disequilibrium on the genes of the regulatory network to detect interactions. In combination, all these approaches will enable us to enlighten the natural variation of the underlying gene regulatory network.

Keywords: TRICHOME, PATTERNING, TTG1, NETWORK, GWAS

**Poster #356. Evolution of the DUF26-containing gene families in plants** (Submission 154)  
Aleksia Vaattovaara\(^1\), Chunxiang Li\(^2\), Ari Loytynoja\(^2\), Jarkko Salojarvi\(^1\), Jaakko Kangasjarvi\(^1\), Michael Wrzaczek\(^1\)  
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Gene family expansions and their relationship to the evolution of gene function over time are of general interest in biology. Plants contain a large number of receptor-like protein kinases (RLKs) which are typically transmembrane proteins (over 600 members in Arabidopsis). One of the subgroups of RLKs are the cysteine-rich receptor-like protein kinases (CRKs). The CRKs form a large plant-specific gene family with 44 members in Arabidopsis and they are involved in plant stress responses and development. However, the discovery of the loss-of-function phenotypes for crk mutants has been difficult due to the high sequence similarity within the CRK gene family, which has been suspected as the main cause for the observed redundancy. Typically, the CRKs consist of an intracellular kinase domain, transmembrane region and an extracellular domain that usually contains two DUF26 (domain of unknown function 26) domains (also known as stress-antifung domain, PF01657). The DUF26 domain contains the conserved cysteine motif C-8X-C-2X-C. DUF26 domains are also found in two closely related gene families, the plasmodesmata-localized proteins (PDLPs) and the cysteine-rich receptor-like secreted proteins (CRSPs). In total, the Arabidopsis genome encodes over 100 DUF26-containing genes. In order to understand their evolution, we have identified CRKs, PDLPs and CRSPs from more than 20 plant species covering most plant lineages. Our main interest is to understand why so many genes in these gene families are maintained after several expansion events and how the variation in the size of the gene family links to changes in development or environmental responses in different plant lineages. We are also aiming to resolve the origin of the DUF26
domain as we have so far identified them only from mosses and higher plants. Our data suggests that genes containing two DUF26 domains appeared for the first time in the lycophytes. Intriguingly, in genes with two DUF26 domains, the first and the second DUF26 domain have differentiated into specific forms with unique sequence context surrounding the conserved cysteines. There is also variation within DUF26 domains between the different phylogenetic subgroups of the CRKs in Arabidopsis. This variation might have functional and structural importance for the extracellular domain of the CRKs.

Keywords: Evolution, Gene family, DUF26, CRK, Cysteine-rich receptor-like protein kinase, Receptor-like protein kinase, RLK

Talk #357. Global genetic heterogeneity for flowering time in Arabidopsis thaliana (Submission 160)
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Using natural variation to explore the genotype-phenotype map is very powerful, and Genome-wide association mapping has become a standard tool to elucidate these relationships. However, depending on trait architecture the number of individuals needed for the analysis can differ dramatically. Additionally selecting a meaningful set of accessions to phenotype is not trivial either, and trait architecture for many traits might be different in diverse geographic regions. Flowering time, for example, shows a clear latitudinal cline and is regulated by umpteen different genes. But are always the same genes responsible for the observed phenotypic differences in distinct geographic locations? And how does the analysis of different sets of accessions affect the power of the analysis? To answer these questions, we phenotyped close to a thousand accessions for flowering time. Due to the progress of the Arabidopsis 1,001 genomes project, full sequence information for these accessions is readily available and more than 6 million markers can be tested in the analysis. This dataset enables an en detail analysis of flowering time in different local and global subsets. Hereby, the results differ dramatically: Many genes are only associated with flowering time in certain subsets. Here we try to understand and explain these differences and present example where the allele frequency of causative alleles differs in different populations, as well as examples, where the actual effect of the respective causal allele differs.

Keywords: Genome-wide association mapping, flowering time, trait architecture

Poster #358. Variation in Arabidopsis flowering time associated with cis-regulatory variation in CONSTANS (Submission 194)
Ulises Rosas¹, Yu Mei², Qiguang Xie³, Josh Banta⁴, Royce W Zhou⁵, Gabriela Seufferheld², C. Robertson McClung³, Michael Purugganan¹, Yoshie Hanzawa²
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The onset of flowering, the change from vegetative to reproductive development, is a major life history transition in flowering plants. Recent work suggests that mutations in cis-regulatory mutations should play critical roles in the evolution of this (as well as other) important adaptive traits, but thus far there has been little evidence that directly links regulatory mutations to evolutionary change at the species level. While several genes have previously been shown to affect natural variation in flowering time in Arabidopsis thaliana, most either show protein-coding changes and/or are found at low frequency (<5%). Here we identify and characterize natural variation in the cis-regulatory sequence in the transcription factor CONSTANS that underlies flowering time diversity in Arabidopsis. Mutation in this regulatory motif evolved recently and has spread to high frequency in Arabidopsis natural accessions, suggesting a role for these cis-regulatory changes in adaptive variation of flowering time.

Keywords: Flowering, Arabidopsis, cis-regulatory variation
Poster #359. Resolving the genetic and epigenetic variations of Arabidopsis with complete genome assemblies (Submission 201)
Chongyuan Luo1, Ronan O’Malley1, Jason Chin2, Alex Hastie3, Paul Peluso2, Weiping Wang3, Heng Dai3, Michael Egholm4, Tim Harkins5, James Knight6, Han Cao2, David Rank2, Joseph Ecker1
1Salk Institute for Biological Studies, United States, 2Pacific Biosciences, United States, 3BioNano Genomics, United States, 4Pall corporation, United States, 5Life Technologies, United States, 6454 Life Science, United States
The landscapes of single nucleotide variants (SNV) and small insertion/deletions across natural Arabidopsis accessions have been comprehensively cataloged by the Arabidopsis 1,001 Genomes Project. The structural variants have remained largely unexplored due to the limitation imposed by genome assembly from short-read sequencing. We resolved the complete genomes of Arabidopsis accessions Col-0, Ler-0 and Cvi-0 with a combination of long-read sequencing and ultra-long-read scaffolding methods. The bridging of Bionano Genome Map and multi-megabase de novo contigs generated from 454 and Pacific Biosciences SMRT sequencing produced chromosome arm length superscaffolds, including extensive coverages of peri-centrometric regions. The de-novo assembly of Col-0 genome resulted 98.3% coverage and greater than 99.99% nucleotide-level accuracy. In addition to confirming over 92% of SNVs identified by Illumina paired-ends sequencing, the de-novo assembly discovered an additional 50% (~250,000) SNV primarily from regions with poor coverage of short-reads. Our reference independent approach allowed the interrogation of regions highly diverged from reference strain Col-0. Sequences showing structure variations between Arabidopsis accessions constitute 13%-14.8% of the genome for each accession. The technical independency and single-molecule read analysis of SMRT sequencing and Genome Map reinforced the validity of structure variants discovered by our method. Thousands of conserved and polymorphic repeats were found between the three Arabidopsis accessions and associated with distinct DNA methylation patterns. Complete de-novo genome assemblies for Arabidopsis accessions provide the critical resource for the comprehensive assessment of interactions between genetic and epigenetic variants.

Keywords: Arabidopsis, structural variation, genome assembly, DNA methylation, epigenetics

Poster #360. Demographic history and genetic diversity of modern and herbarium collections of Arabidopsis thaliana from the Southern Hemisphere (Submission 218)
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The model plant, Arabidopsis thaliana, has a native range throughout Eurasia and North Africa and introduced populations have been found on several additional continents. Through the RegMap and 1001 Genomes projects, high resolution genetic data has been obtained for thousands of A. thaliana populations that are mostly distributed throughout the Northern Hemisphere. To gain insight into the genetic variation and demographic history of introduced populations of A. thaliana in the Southern Hemisphere, we collected plants from four ecologically diverse sites at the southernmost end of the species distribution in Patagonia, Argentina. Two of the sites were spaced 5 km apart on the south side of Lake Buenos Aires and two neighboring sites were selected 70 km away on the north side of the lake. Individuals collected exhibited a wide range of phenotypic characteristics. We analyzed the genetic diversity by low-resolution SNP genotyping and whole-genome sequencing. We also performed whole-genome sequencing of the DNA from an herbarium specimen collected from this region in 1967. We found that individuals from all four present-day populations were essentially genetically uniform across 149 SNP markers. Whole-genome sequencing revealed that only a small fraction of the genome was segregating among these strains. Genetic comparisons with the RegMap panel and 1001 Genomes data suggested a likely European origin for these populations. Sequencing data from the herbarium specimen revealed that the present-day populations are essentially the same as they were nearly half a century ago. We conclude that populations of A. thaliana in Patagonia were likely founded by an extremely small number of individuals and that phenotypic plasticity may have contributed to their persistence in the region despite their limited genetic diversity.

Keywords: Natural variation, Next-generation sequencing, Population genetics, ancient DNA sequencing, Ecology, Evolution, Phenotypic plasticity
Poster/Talk #361. The origin of the plant hormone ethylene predates the colonization of land (Submission 241)
Bram Van de Poel1, Chuanli Ju1, Endymion Cooper1, James Thierer1, Ted Gibbons1, Charles Delwiche1, Caren Chang1
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The plant hormone ethylene is well characterized in land plants, particularly in angiosperms. Its biosynthesis route is known, the signaling pathway has been dissected, and many physiological responses are described. Yet the origin and evolution of ethylene are unknown. When during evolution did ethylene become a functional hormone? Ethylene is known to operate as a hormone in all embryophytes (land plants), but little is known about ethylene in algae. Land plants evolved over 450 million years ago from the charophytes, which are freshwater green algae. By investigating charophyte green algae, which are the closest living ancestors of land plants, we obtained evidence that ethylene was already a functional hormone 450 million years ago. By de novo transcriptome sequencing of five representative species of the charophyte algae, we retrieved homologs of several hormonal pathways including those for ethylene, auxins, abscisic acid, cytokinins and brassinosteroids. In particular, homologs for the entire ethylene biosynthesis and signaling pathways were found in the filamentous charophyte Spirogyra pratensis. Focusing on ethylene, we were able to show that Spirogyra is capable of producing ethylene. Interestingly ethylene induces a striking cell elongation response in Spirogyra, possibly representing an ancestral ethylene response. Due to the lack of a genetic methods for Spirogyra, we employed Arabidopsis to investigate the ethylene signaling pathway of Spirogyra. By expressing Spirogyra ethylene homologs in well-defined Arabidopsis ethylene mutants, we were able to show that the ethylene signaling pathway in Spirogyra is homologous to that in Arabidopsis. We also showed that post-translational modifications and protein interactions of certain ethylene signaling components of Spirogyra were conserved in Arabidopsis. Our results demonstrate that ethylene is a functional hormone in Spirogyra and indicate that the common aquatic ancestor of this charophyte green alga and land plants already possessed all the machinery for ethylene production and response, and thus ethylene was most likely a plant hormone prior to the colonization of the land.

Keywords: Plant Hormone Evolution, Ethylene, Charophytes, Algae

Poster #362. Frequent changes in expression profile and accelerated sequence evolution of duplicated imprinted genes in Arabidopsis (Submission 315)
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Eukaryotic genomes have large numbers of duplicated genes that can evolve new functions or expression patterns by changes in coding and regulatory sequences, referred to as neofunctionalization. In flowering plants, some duplicated genes are imprinted in the endosperm, where only one allele is expressed depending on its parental origin. We found that 125 imprinted genes in Arabidopsis arose from gene duplication events during the evolution of the Brassicales. Analyses of 46 gene pairs duplicated by an ancient whole genome duplication (alpha WGD) indicated that many imprinted genes show an accelerated rate of amino acid changes compared to their paralogs. Analyses of microarray expression data from 63 organ types and developmental stages indicated that many imprinted genes have expression patterns restricted to flowers and/or seeds in contrast to their broadly expressed paralogs. Assays of expression in orthologs from outgroup species revealed that some imprinted genes have acquired an organ-specific expression pattern restricted to flowers and/or seeds. The changes in expression pattern and the accelerated sequence evolution in the imprinted genes suggest that some of them may have undergone neofunctionalization. The imprinted genes MPC, HDG6 and HDG3 are particularly interesting cases that have different functions from their paralogs. This study indicates that a large number of imprinted genes in Arabidopsis are evolutionarily recent duplicates and that many of them show changes in expression profiles and accelerated sequence evolution. Acquisition of imprinting is a mode of duplicate gene divergence in plants that is more common than previously thought.

Keywords: duplicated genes, whole genome duplication, imprinted genes, neofunctionalization
Poster #363. GENETIC ARCHITECTURE OF PARENTALLY-BIASED SEED SIZE DETERMINANTS (Submission 369)
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In Arabidopsis, parental genomic imprinting (PGI) has been characterized in the seed endosperm, which provides maternal resources akin to the mammalian placenta. Loss of PGI in DNA methyltransferase mutants produces large or small seeds if the mutation is inherited maternally or paternally, respectively. Although the mechanisms for imprinted gene expression have been characterized extensively in select genes, less is known about the adaptive role or utilization of imprinted gene programs in wild-type plants. It is expected that PGI could lead to parent-of-origin effects or specific maternal/paternal interactions that might be masked as a component of heterosis under traditional breeding schemes. Understanding the developmental programs regulated by PGI may therefore provide clues to optimizing breeding strategies for seed size. We sought to identify cellular events regulated by imprinted gene expression programs in Landsberg erecta (Ler) and Cape Verde Islands (Cvi) ascensions. A parental bias is observed in these lines with Ler fathers promoting a transgressive large seed not seen in the reciprocal cross. Using spectral analysis of autofluorescence in the seed, we isolated endosperm from surrounding maternal structures in these non-transgenic natural variants. Reconstructed 3D endosperm models show that Cvi development involves an adaxial-abaxial expansion not present in Ler, which restricts expansion on this axis maternally. Ler fathers, however, promote an anterior-posterior expansion. We are using the Cvi x Ler recombinant inbred line population to isolate loci responsible for the maternal and paternal components of this phenomenon.

Keywords: Recombinant inbred lines, Natural variation, seed development, parental conflict

Poster #364. To Flower or Not to Flower (in Maine)? (Submission 425)
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Arabidopsis thaliana, while native to Europe and Asia, has been introduced to North America. It has been recorded only once in the state of Maine in Cumberland County, but is found widely in other parts of the midwest, west and Canada. Northern Maine appears to be just beyond its range. To give us an idea about the genes involved in this range restriction and about possible range expansion potential due to climate change, we explored the flowering behavior of a variety of genotypes of A. thaliana planted in Presque Isle, ME on the University of Maine at Presque Isle campus and documented their flowering times when planted at intervals though the summer of 2013. The vernalization gene FRIGIDA played an important role in controlling the timing of flowering, but the magnitude of its influence varied depending on the planting date. While early plantings were somewhat successful, later planting were not, and no plants were found to survive to overwinter as might have been expected, including an ecotype from Finland. Heavy rains were found to severely impact the growth of young seedlings and thus might influence the range restriction of A. thaliana in Maine.

Keywords: range expansion, flowering time, adaptation, natural variation

Novel Tools and Techniques

Poster #365. ePlant: A user friendly data visualization tool for integrating and exploring multiple levels of biological data. (Submission 13)
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The Arabidopsis community has generated numerous large data sets for exploring multiple aspects of plant biology. Recent efforts to bring together several of these data sets are underway in the form of the Arabidopsis Information Portal. In order to foster the creative processes that lead to better hypothesis generation, researchers need user friendly tools that facilitate easy filtering, browsing and analysis of multiple levels of data at the same
time. Aggregating multiple levels of data in a well organized portal should improve research work flows, but aggregation is only the first step towards actual integration. We present here a zoomable user interface that enables seamless browsing, exploration and comparison between multiple large data sets, organized into an intuitive hierarchy of interrelated biological levels of analysis. Taking into account the latest research on data visualization, preattentive processing and rapid serial visualization, ePlant was designed to help researchers make connections between biological levels of organization from macro to micro all on the same screen.

ePlant currently contains data visualization modules for interactive exploration of natural variation and environmental factors, cell and tissue expression patterns, protein-protein interactions, transcriptome responses to various experimental conditions, 3D protein models, and a genome browser for exploring gene features and nucleotide sequence information. Dynamic links to the underlying data, annotations and other information are available on demand. The code is open source and well documented to ensure transparency and accuracy of the information reported. The system was designed to facilitate future expansion and we have plans to include additional species, data sources and new data visualization modules in our next release. We are actively soliciting feedback to guide future development of this tool. This conference provides an opportunity to collect qualitative data from the very population of users that the tool is being developed for. The closing portion of our presentation will focus on this evaluation effort, with the goal of encouraging potential users to help us determine our next priorities to better serve the needs of the Arabidopsis community.

ePlant can be found at: http://bar.utoronto.ca/eplant

Keywords: Data visualization tool, Hypothesis generation, Integrative system, Zoomable user interface

Talk #366. Unraveling cell proliferation control in plants through functional interactomics: from cells to tissues. (Submission 23)
Jelle Van Leene1, Maarten Dedecker1, Nienke Besbrugge1, Astrid Gadeyne1, Daniel Van Damme1, Liesbeth Vercruysse1, Hilde Nelissen1, Aurine Verkest1, Dominique Eeckhout1, Geert Persiau1, Eveline Van de Slijke1, Nancy De Winne1, Bernard Canoot1, Leen Vercruysse1, Kris Gevaert1, Dirk Inze1, Geert De Jaeger1
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Our research team has fine tuned a versatile TAP technology platform for protein complex isolation from both Arabidopsis cell cultures and whole seedlings. We isolated complexes for hundreds of proteins involved in cell cycle control and phytohormonal pathways and extensively demonstrated the power of our technology for functional analysis of proteins and protein complexes, and the mapping of protein networks in plant research. More recently we focused on the functional analysis of protein complexes involved in cell division plane determination and cell proliferation control in Arabidopsis thaliana. A TTP complex was discovered and shown to be involved in spacial control of plant cell division (1). A T-plate complex involved in cell plate formation turned out to be a plant specific complex regulating endocytosis (2). And the regulator of cell proliferation AN3 was shown to be part of a SWI/SNF chromating remodelling complex involved in leaf size determination (3). The next step we are currently taking is the study of protein complex function and dynamics involved in leaf organ size control. Therefore, we successfully transferred our technology to corn and obtained proof of concept of protein complex dynamics during leaf development.
(3) Vercruysse et al. (2014) Plant Cell 26, 210-29.

Keywords: Tandem affinity purification, Protein complex, Protein interactions

Talk #367. Gamma-ray imaging system for studying heavy metal transport in plants (Submission 95)
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Heavy metal hyperaccumulation in plants is an adaptation to metalliferous soils and allows the plants to uptake high concentrations of the typically toxic compounds. Understanding the mechanism of metal uptake and accumulation is of great interest for the generation of plants to be used for phytoremediation of toxic soils. Current
methods of studying metal migration in plants are limited in their availability (synchrotron x-ray fluorescence), scale (e.g. confocal microscopy of FRET sensors), or dynamics (e.g. quantification of metals accumulated by tissue). We are presenting a new method that allows dynamic imaging of whole-plant metal transport and sequestration. We have developed a custom built, high-sensitivity single photon emission computed tomography (SPECT) imaging system. The imaging system is suited for Arabidopsis-sized plants in liquid growth media and uses radioisotope forms of metals to study in planta metal transport and accumulation. The SPECT detects the gamma-ray emissions of the radioisotopes, e.g. 109Cd (22 keV) or 65Zn (1.1 MeV), and as it is able to discriminate between these signals, the effect of any competition between the metals in the uptake mechanism can also be studied. As an example study, we imaged the transport of Zn in whole plants of heavy metal non-accumulator Arabidopsis thaliana and metal hyperaccumulator Arabidopsis halleri to elucidate the differences in the dynamics of heavy metal uptake and translocation between these closely related species. Additionally, we imaged Zn transport in Arabidopsis halleri HMA4-RNAi line, where heavy metal transporter HMA4 is down-regulated, to test the effect of an individual gene on the uptake and translocation dynamics. To our knowledge, this is the first attempt to investigate 65Zn uptake and root-to-shoot translocation rates with nuclear imaging.

Keywords: heavy metal accumulation, Arabidopsis halleri, nuclear imaging

Poster #368. An affinity-based target identification approach for a small molecule inhibitor of endocytosis in Arabidopsis (Submission 103)

Wim Dejonghe, Kiril Mishev, Evelien Mylle, Anna-Maria Szatmari, Bram Denoo, An Staes, Daniel Van Damme, Annemieke Madder, Kris Gevaert, Johan Winne, Natasha Raikhei, Eugenia Russinova

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Signaling induced by receptor-ligand interaction, such as the recognition of brassinosteroids by the leucine-rich repeat receptor BRASSINOSTEROID INSENSITIVE1, can immediately occur from the plasma membrane, or might require internalization of the ligand-receptor complex into endosomes, through endocytosis, where it meets the required components to initiate signaling. Conversely, uptake of the receptor-ligand complex can attenuate signaling. The brassinosteroid signaling pathway is an excellent model to study the influence of endocytosis on ligand-receptor complex mediated signaling, as most of the signaling pathway is known. With the help of a fluorescently labeled brassinosteroid, both pharmacological and genetic showed that the main pool of active BRI1 resides at the plasma membrane, while clathrin-mediated endocytosis serves as an attenuation mechanism. Because signaling is governed by a highly dynamic process such as endocytosis, classical genetics strategies are less suited to get a better understanding of molecular components and mechanisms involved. A possible solution to this problem lies in a chemical genetics strategy, where one seeks the molecular target for a small molecule inducing a phenotype of interest. Here, we screen for small molecules affecting both endocytosis and brassinosteroid signaling, and set out to identify the molecular targets of hit molecules. Target identification of hit molecules is based on affinity purification, and subsequent mass spectrometry analysis.

Keywords: Endocytosis, Chemical genetics, affinity purification, Target identification

Poster/Talk #369. Inverting E3 Ligase Function in the Circadian Clock Using a Decoy Strategy (Submission 120)

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The circadian clock is a critical endogenous timing mechanism that integrates environmental information to assign biological processes to specific times of day. All clocks have a conserved architecture that includes inputs that provide time-of-day information, the clock mechanism itself, and outputs that are biological processes parsed to specific times. In plants most studies have focused on the transcriptional feedback loops that make up the core
clock and connect to outputs by controlling gene expression. Little is understood about the mechanisms that control rhythmic protein degradation that is critical to oscillating protein abundance of clock components and factors involved in output processes. Our lab is utilizing a "decoy" strategy to understand the genetic role of F-box containing E3 ligase proteins in the rhythmic degradation of clock proteins and to define F-box interactomes to identify the full suite of substrates and interacting partners. We have created affinity tagged dominant negative versions of the E3 ligases that regulate the stability of critical clock factors. This allowed us to probe the genetic role of these proteins in spite of gene redundancy. Additionally, the normally unstable target substrates are now stabilized allowing for identification by IP-mass spectrometry. Initial genetic and mass spectrometric results will demonstrate the robustness of the decoy technique in defining function and identifying previously elusive substrates and interaction partners. Determining how protein stability is rhythmically controlled will move the circadian field vertically by expanding our understanding of how the clock integrates and responds to environmental information at the post-translational level.

Keywords: Ubiquitin Proteasome System, Circadian Clock, Biochemistry, Genetics

Poster #370. High-power genetic mapping using target-enrichment sequencing (Submission 130)
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Complex traits such as crop performance and human diseases are often controlled by multiple genetic loci with small contributions, which are not readily detectable by traditional QTL (quantitative trait locus) mapping using small populations. Bulk segregant analysis with large F2 pools and genome-level markers (named extreme QTL or X-QTL) can dissect comprehensive genetic structures underlying complex traits. However, simulations suggest that greater than 1000X sequencing depth is needed to maximize QTL detection for bulk segregant studies with large F2 populations. For most organisms, whole genome sequencing at such depth is not economically feasible. Here we show that target enrichment to capture and sequence markers can provide the sequencing depth needed for maximizing QTL detection. We combined theoretical and empirical approaches to demonstrate the efficacy and power of this strategy by mapping QTLs for early seedling development under salt stress in Arabidopsis thaliana. Incorporating target enrichment into QTL mapping mitigates the effect of the genome size on achievable sequencing depth. We anticipate that our approach will expedite the pace of QTL identification and the dissection of genetic architectures underlying complex traits for many organisms.

Keywords: high-power QTL mapping, target-enrichment sequencing, detection of small-effect QTL

Poster/Talk #371. Protein-ligand Interaction Exploration Based On Proteome-wide Tertiary Structure Prediction and Further in vitro Validation (Submission 166)
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Proteins rarely function alone, most of the times they interact with the other molecules to fulfill their functions. Protein-ligand interactions are crucial for biological activities and the exploration and analysis of protein-ligand interactions are essential for our biological understanding of cellular function. In my study, I will use bioinformatics methods to screen for candidate proteins that can bind to certain ligands (carbohydrates and proteins) based on their structural features such as aromatic side chain shapes and protein-protein surface shapes (shape complimentarity) from a protein structure-ome containing 67,275 predicted protein structure models covering around 70% of the Arabidopsis proteome (Fucile et al., 2011). The predicted interactions will be further validated using in vitro methods. My project aims to (1) provide evidence that predicted protein structures can be used in protein structure and functional studies, and (2) explore the relationship between protein structure and protein-ligand interaction ability.

Keywords: Predicted Arabidopsis Structure-ome, Protein-carbohydrate interaction, Protein-protein interaction
**Poster #372. Green to Red Photoconversion of Wild Type EGFP as a Simple and Powerful Tool for Investigating Protein and Organelle Dynamics** (Submission 175)
Amirali Sattarzadeh¹, Reza Saberianfar², Warren R. Zipfel³, Rima Menassa⁴, Maureen R. Hanson⁵
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Fusions of Green Fluorescent Protein (GFP) and its derivatives are extremely valuable tools for examining gene expression, protein and RNA localization, protein-protein interactions, protein synthesis and degradation, and organelle movement. The development of special photoconvertible fluorescent proteins allows new questions to be asked concerning dynamic processes in living cells. However, use of some photoconvertible proteins has some drawbacks. For example, non-GFP photoconvertible fluorescent proteins sometimes exhibit unexpected subcellular localization behavior when fused to other proteins and irradiated. Another issue concerning their use is the necessity to introduce new transgenes expressing the photoconvertible proteins into the genotypes of interest. Because many proteins have already been tagged with EGFP in transgenic plants, being able to photoconvert EGFP would allow use of existing transgenic lines for studies of dynamic changes. In low oxygen or in the presence of various electron acceptors, irradiation with blue light has been reported to transform GFP from the green to the red fluorescent state. Here we report that GFP can be efficiently photoconverted in vitro without adding either electron acceptors or reducing oxygen. We also examined whether EGFP could be converted from the green to the red state in vivo. We will describe an efficient method for EGFP photoconversion for ultrafast, regional and non-invasive marking of proteins in vivo in plant cells. Using time-lapse confocal microscopic movies, we will present several examples that demonstrate that EGFP can be photoconverted from green to red in transgenic cells of Arabidopsis and Nicotiana for observation of protein dynamics. For example, converting EGFP to red-EGFP results in rapid diffusion of the red form within the plant cytoplasm.

Keywords: Green Fluorescent Protein, GFP, Photoconversion, Organelle, Confocal Microscopy

**Poster/Talk #373. Mn-eyevering Manganese: using Synchrotron X-Ray Fluorescence on living plant tissue to understand metal mobilization** (Submission 192)
Amanda Socha¹, Tracy Punshon¹, Mary Lou Guerinot¹
¹Dartmouth College, United States

Manganese (Mn) is an essential trace element that is required for plant health. In plants, Mn serves as a cofactor in essential processes such as photosynthesis, lipid biosynthesis and oxidative stress. However, in high quantities, Mn can be toxic. Therefore, maintaining proper Mn homeostasis is necessary for optimizing crop production. Multiple transport proteins have been identified that are required for Mn transport, in particular those belonging to the NRAMP (natural resistance associated macrophage protein), MTP (metal tolerance protein) and ZIP (zinc regulated transporter/iron-regulated transporter related protein) families. However, the significance and interplay of these transporters in long distance transport and trafficking into and out of organelles is not fully understood. We are using the powerful tool of Synchrotron X-ray Fluorescence Spectroscopy (SXRF) microanalysis, which allows non-destructive multi-elemental imaging of live tissues without the need for sample preparation or sectioning. We are now capable of imaging tissues at sub-micron resolutions. This allows us to explore how patterns of Mn localization are set up and maintained by imaging metal distribution during dynamic processes such as germination. We previously demonstrated that Mn is localized in the sub-epidermal cell layer of embryonic cotyledons. By examining developing seedlings, we see that this pool of Mn is quickly re-distributed in young cotyledons. We are now investigating which transporters are responsible for setting up these patterns. We are also exploiting natural variation in Mn concentration in Arabidopsis accession lines to identify genes involved in Mn homeostasis. The ultimate goal of this project is to understand how plants acquire, transport and mobilize Mn. By better understanding how plants take up and utilize essential nutrients, we can take steps towards sustainable improvements in crop yields both in terms of plant productivity and nutrient content, contributing to food security and improved human nutrition.
Keywords: Synchrotron X-Ray Fluorescence, Manganese, metal transport

**Poster #374. A novel method to understand short tandem repeat variation and function in Arabidopsis thaliana** (Submission 197)
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Short tandem repeats (STRs), repetitive DNA sequences with unit size from 2-10 base pairs, have extremely high mutation rates due to polymerase slippage during DNA replication. Resultant STR variation in coding and regulatory regions can significantly affect phenotype and based on a few examples, such variation is thought to facilitate rapid adaptation to new environments. Additionally, the high mutation rate of STRs allows them to be sensitive markers of genomic instability within an individual. A systematic assessment of STR variation and function has been hindered by the fact that STRs are not readily accessible by high-throughput, short-read sequencing. In response to this, we developed a novel method combining single molecule capture and deep sequencing to accurately assess variation in STR unit copy number, both germline variation across individuals and somatic variation within individuals. Amplification and sequencing of repeats introduce rampant technical error. Despite these technical obstacles, our single molecule method allows us to precisely determine the germline allele and to distinguish between technical error and true somatic mutation events. In our pilot study, we called genotypes of 100 STR loci across 96 diverse strains of Arabidopsis thaliana with greater than 95% accuracy. We find loci which are more or less variable across 96 strains than predicted by sequence characteristics, which may indicate these STRs are under either diversifying or purifying selection respectively. We also measured levels of genomic instability in different mutants and genetic backgrounds to further our understanding of the role of genomic instability in organismal phenotype. Our novel method can be applied broadly to assess STR variation genome-wide in any sequenced organism.

Keywords: short tandem repeats, molecular inversion probes, degenerate sequence tags, natural variation, somatic variation

**Talk #375. Advances in crossover localization and QTL mapping using high-throughput sequencing.** (Submission 220)
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Crossovers (COs) that form between homologous chromosomes during meiosis allow for novel combinations of alleles in progeny and can therefore generate trait variation that did not exist in the parental generation. The frequency and spatial location of COs are not only important for generating the raw material for evolution, but also contribute to our ability to perform quantitative trait locus (QTL) mapping for phenotypic traits. Understanding the mechanisms that govern CO formation will inform our current views of its importance for evolution and also provide potential targets for facilitating plant breeding. Engineering changes in the frequency and distribution of COs can improve QTL mapping of important agricultural traits and an also allow for more efficient trait introgression by reducing the number of linked loci. Here we describe improvements in the ability to precisely locate crossover events and perform QTL mapping using low-coverage whole-genome sequencing of individuals in mapping populations. We developed a method for preparing libraries for next-generation sequencing at about a tenth of the cost of established protocols and then used a refined Hidden Markov Model approach to reconstruct the recombinant genomes of hundreds of F2 individuals generated from the Col-0 and Ws-2 accessions of Arabidopsis thaliana. We resolved most CO breakpoints to within 2 kb and detected a 25-kb QTL interval for flowering time on Chromosome 5 that includes the MAF2-5 gene cluster, which has previously been shown to contribute to flowering time variation in natural populations. We conclude that this approach is efficient for studying genetic factors affecting the crossover landscape and allows for the simultaneous (fine-) mapping of quantitative traits.

Keywords: Next-generation sequencing methods, Hidden Markov Model, Genome reconstruction, QTL mapping, meiosis, recombination
Poster/Talk #376. RAPA: RaspberryPi Automated Phenotyping Array provides low-cost, scalable, automated, high-throughput, in-place phenotyping (Submission 222)
George Wang¹, Andre Noll², Christian Widmer¹, Beth Rowan², Detlef Weigel³
¹Max Planck Institute for Developmental Biology, Germany, ²Max Planck Institut for Developmental Biology, Germany, ³MPI for Developmental Biology, Germany
Today, genetic data is relatively cheap, but phenotypic data is costly in time and resources. Many phenotypes are measured by hand. The full potential of next-generation sequencing will not be realized without high-throughput phenotyping. Several high-throughput phenotyping solutions exist, but they are expensive and often require large infrastructure investments. Here, we present RAPA: the RaspberryPi Automated Phenotyping Array. The system consists of one to arbitrarily many RaspberryPi computing units and cameras, backed by a unified system for control, monitoring, and maintenance. Plants are imaged simultaneously, in place, without handling. Images are then automatically segmented using machine learning based software, and morphometrics are extracted and placed in a web-accessible database. RAPA provides a complete consistent photographic record of all plants monitored, in addition to a unified database of experimental metadata and phenotypic outcomes. RAPA is constructed using open source software, and full specifications will be made available.

Keywords: Automated Phenotyping, Image analysis, Machine Learning

Poster #377. A high-throughput and high-resolution dynamic imaging system and its application in studying hormone/light-regulated hypocotyl and root growth of seedlings (Submission 471)
Yusi Ji¹, Hongwei Guo¹
¹Peking University, China
Dynamic imaging provides a novel perspective to reveal detailed and real-time growth processes that may fail to be resolved in end-point static phenotype studies. Existing imaging methods were limited to either low-throughput experimental configurations, or insufficient analyzing precision. We developed an imaging system to analyze the kinetics of hypocotyls and roots of Arabidopsis seedlings, which settled the above concerns of limitations. This system was able to capture 50~100 seedlings in a run of 5-minute interval, with a spatial resolution of ~1.5 mm/pixel. 940 nm infrared illuminating source made it possible to image etiolated seedlings. Multiple treatments, such as mono-color lights or different concentrations of gas mixture, were available by pre-programmed automatic control. A supporting image processing algorithm was developed in Matlab to analyze the growth rate of hypocotyls and roots. Local image alignment using feature extraction and geometric transformation was applied to automatically determine the terminal points when measuring the length of hypocotyls, which improved the robustness and the accuracy significantly in comparison with all reported methods. Taking advantages of the new system, we are investigating the rapid ethylene response of seedlings and light-triggered hook opening, both of which have provided exciting findings and showed the irreplaceability comparing to other experimental approaches. Data retrieved in the experiments can also be analyzed for modeling or genome-wide association study.

Keywords: dynamic imaging system, growth kinetics, rapid ethylene response, hook opening

Plant Hormones

Poster #378. Evolution of strigolactone-specificity in the karrikin receptor KAI2 is a likely basis for host-recognition in parasitic plants (Submission 33)
Caitlin Conn¹, Drexel Neumann¹, Dave Nelson¹
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Strigolactones have recently been recognized as plant hormones with roles in axillary branching, root architecture, secondary growth, and arbuscular mycorrhizal symbioses. However, strigolactones were first identified over 50 years ago in root exudates as potent germination stimulants of the parasitic plants witchweed and broomrape. These parasites are noxious weeds that annually cause billions of dollars in crop losses and impact millions of smallholder farmers in Africa; understanding how these parasites detect host-derived
strigolactones may enable innovative approaches to combat their spread. Rapid advances have been made in our understanding of strigolactone biosynthesis and signaling thanks to genetic studies performed in Arabidopsis, rice, and petunia. We have harnessed these discoveries and the power of Arabidopsis as a genetic tool to investigate how parasites sense their hosts.

In Arabidopsis two homologous a/b-hydrolase superfamily proteins, KAI2 and D14, are key components of strigolactone and karrikin signal transduction. KAI2 is necessary for promotion of seed germination by karrikins (a class of germination stimulants found in smoke), whereas D14 is required for control of axillary shoot branching by strigolactones. Recent biochemical and crystallographic evidence strongly favor the idea that KAI2 and D14 act as receptors. We hypothesized that host recognition could have arisen in parasites if KAI2 evolved strigolactone-specificity. We performed a molecular evolutionary analysis of KAI2 and D14 orthologs in several parasitic species. In comparison to non-parasitic angiosperms, KAI2 in parasites has undergone atypical gene duplication and faster rates of protein evolution. Structural modeling suggested that a set of fast-evolving KAI2 paralogs have D14-like ligand-binding pockets. We used cross-species complementation analysis to test whether KAI2 paralogs from two parasite species can restore the germination and seedling growth responses to karrikin or strigolactone in the Arabidopsis kai2 mutant. Our experiments demonstrate that a set of fast-evolving KAI2 paralogs have gained strigolactone-specificity.

Keywords: strigolactone and karrikin signaling, germination, evolution

Talk #379. Identification and characterization of the polarly-localized TRANSPORTER OF IBA1 (Submission 57)
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Levels of auxin, which regulate both cell division and cell elongation in plant development, are controlled by synthesis, inactivation, transport, and the use of storage forms. Conversion of the auxin precursor indole-3-butyric acid (IBA) to the active auxin indole-3-acetic acid (IAA) contributes to an array of seedling development events, including cotyledon expansion, root hair elongation, lateral root formation, apical hook formation, and root meristem maintenance. Although IBA transport mechanisms appear to be independent of IAA transport mechanisms, carriers required for IBA movement across the plasma membrane remain largely unidentified. The ATP-binding cassette (ABC) transporter ABCG36 is necessary for IBA efflux and abcg36 mutants are hypersensitive to the effects of IBA on root elongation inhibition. To identify IBA uptake carriers, we performed an abcg36 suppressor screen, hypothesizing that decreasing IBA uptake would suppress the abcg36 hypersensitivity resulting from blocked IBA efflux. In this screen, we identified a mutation in the founding member of an uncharacterized Major Facilitator Superfamily clade of transporters, which we named TRANSPORTER OF IBA1 (TOB1). Intriguingly, TOB1 displays a unique polar localization within several tissues, and its expression is coordinated with sites of IBA-to-IAA conversion, suggesting that TOB1 plays roles in regulating conversion of IBA to active auxin. Our characterization of tob1 reveals the complex nature of regulating levels of IBA and IBA-derived auxin within the cell to drive developmental processes.

Keywords: auxin, transport mechanisms, IBA, polar localization

Poster #380. The function of EIN2 in the nucleus (Submission 61)
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EIN2 is the essential key regulator of ethylene signaling pathway, and it is the only gene whose null mutant is completely ethylene insensitive. Specifically, EIN2 is highly conserved through 372 plant species from lower plants to higher plants including many important crops. Recently, our study and those of others have uncovered a mechanism whereby EIN2 protein processing and subcellular nuclear translocation are required for the response to ethylene. Furthermore, ethylene triggers dephosphorylation at several sites and proteolytic cleavage at one of these sites, resulting in nuclear translocation of a carboxyl-terminal EIN2 fragment, leading to the EIN3/EIL1-dependent transcription and the activation of an ethylene response. The new studies open a new avenue of
ethylene signaling pathway. Our study is focus on the function of EIN2 C-terminal end in the nucleus.

Keywords: ethylene signaling, EIN2, nucleus

Poster #381. A novel thioredoxin reductase activity of Arabidopsis YUCC6 confers drought tolerance independently of auxin biosynthesis (Submission 63)
Joon-Yung Cha¹, Woe-Yeon Kim¹, Dae-Jin Yun¹
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YUCCA (YUC) proteins constitute a family of flavin monooxygenases (FMOs) with an important role in auxin (IAA) biosynthesis. We report that Arabidopsis plants overexpressing YUC6 display enhanced IAA-related phenotypes and exhibit improved drought stress tolerance, low rate of water-loss and controlled ROS accumulation under drought and oxidative stresses. Co-overexpression of an IAA conjugating enzyme reduced IAA levels but drought stress tolerance was unaffected, indicating that the stress-related phenotype is not based on IAA overproduction. We show that YUC6 contains a previously unrecognized thioredoxin reductase (TR) domain that overlaps with the FMO domain involved in IAA biosynthesis. Mutation of a conserved cysteine residue (Cys-85) suppressed TR but preserved FMO activity, whereas mutating the FAD and NADP binding sites, that are common to TR and FMO domains, abolished both activities. We provide a paradigm for a single protein playing a dual role, regulating plant development and conveying stress defense responses.

Keywords: auxin, YUCCA6, thioredoxin reductase, reactive oxygen species, drought tolerance, flavin monooxygenase

Poster #382. Generation of a superactive MYC2 transcription factor to increase the jasmonate response in plants (Submission 69)
Jonas Goossens¹, Laurens Pauwels¹, Robin Vanden Bossche¹, Alain Goossens¹
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Jasmonate (JA) is an important phytohormone involved in several developmental processes and in plant defense, activating the production of specialized metabolites. Many of these bioactive compounds possess important characteristics for potential use in pharmacological or industrial applications. The path towards JA-elicited production of specialized metabolites, consists on the one hand of a very conserved primary signaling pathway and on the other hand of a more species-specific specialized metabolism, involving biosynthetic enzymes. The central bridge between the primary and specialized pathway is generally regulated by transcription factors which steer expression of e.g. different enzymes involved in a particular metabolic biosynthetic branch. In the JA signal transduction cascade this is carried out by the MYC transcription factors, in particular by MYC2. All together this makes transcription factors attractive targets for metabolic engineering in plants. Overexpression of endogenous transcription factors is however often not sufficient to increase the downstream response, for instance due to complex post-translational regulation. Here we generated a superactive Arabidopsis MYC2, SuperMYC, by altering its amino acid sequence, modulating its regulation. MYC2 interacts with the family of the JAZ repressors. SuperMYC was able to escape from interaction with nearly all JAZ proteins, leading to increased transactivation activity. Furthermore, overexpression of SuperMYC in Arabidopsis resulted in a hypersensitive JA phenotype. Deeper insight in the interaction between SuperMYC and JAZ revealed the possible existence of functional non-redundancy among the family of JAZ repressors. Our results underscore the possibility of creating superactive transcription factors for metabolic engineering programs by modulating their regulation and as a consequence enhancing the induction of the downstream response in plants. The fact that central transcription factors like MYC2 have a conserved function in the plant kingdom might allow these superactive transcription factors to be used for metabolic engineering of medicinal plants.

Keywords: Jasmonate, Specialized metabolites, Protein-protein interactions, Superactive transcription factors

Poster #383. Regulation of cytokinin biosynthesis in response to nutritional cues to optimize growth and development (Submission 75)
Hitoshi Sakakibara¹, Takatoshi Kiba¹, Mikiko Kojima¹

Keywords: cytokinin, nutritional cues, growth, development
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Cytokinin (CK) plays an important role in regulation of plant growth and development, and its action is finely controlled by various steps including biosynthesis and metabolism, transport, and signaling. Previous studies have shown that IPTs, CYP735As, and LOGs are expressed in various parts during growth and development, and differentially regulate the synthesis of N6-[(2R)-2-isopentenyl]adenine (iP) and trans-zeatin (tZ). Detailed studies on CYP735As mutants show that tZ is important for the normal growth of shoot rather than that of root, suggesting a mechanism that modulates physiological function of CKs by modification of the side-chain structures.

As for regulation of CK action by environmental cues, it is shown that CK is a signal molecule propagating N signals throughout the whole plant body for linking N nutrition status to growth regulation, and previous studies have shown that tZ accumulation level is enhanced in response to N availability in various plants. Our recent studies show that there is dual regulation system of AtIPT3 expression in response to nitrate ion and glutamine metabolism, as external and internal N environmental cues, suggesting that plants possess multiple regulation systems to modulate growth in response to N availability.

On the other hand, it is known that higher CO2 environment enhances plant growth and development, but the role of phytohormones in the growth regulation is poorly understood. Our recent studies revealed that specific CK biosynthesis genes including CYP735A are up-regulated in response to higher CO2 condition, and sugar derived from photosynthesis is a key signal of the regulation.

We will outline our recent progress in CK biosynthesis and its regulation in response to N and C availability, and discuss the physiological significance of regulation of CK action to optimize growth and development at whole plant level.

Keywords: Arabidopsis, Biosynthesis, Carbon, Cytokinin, Long-distance translocation, Nitrogen

Poster #384. Repetitive auxin response remolds AUX/IAAs-ARFs-Tir1/AFBs mediated feedback system. (Submission 82)
Masaaki Watahiki

Hokkaido University, Faculty of Science, Japan

Phytohormone auxin controls diverse developmental pathway in plants. For example, the machinery of lateral root formation proposed stepwise progress of developmental stages. Auxin contributes to each its steps forward (Fukaki and Tasaka, 2009). The development of organ or tissue specification often accompanied with subsequent cell division for morphogenesis. An auxin maximum which is local accumulation of auxin precedes lateral root initiation in xylem pole pericycle cell or leaf primordial formation in shoot apical meristem (Bainbridge et al., 2008) and the position of the maximum will be changed to next loci of organ formation accompanied with cell division. With the change of a stand point, the loci of organ formation will experience the transient activation of auxin signaling and desensitizing for auxin. Subsequent developmental sequence continues and auxin also contributes its processes as mentioned above. Such a stepwise development with activation of auxin signaling implicates stepwise remodeling of auxin signaling during plant organogenesis. Actually oscillation of auxin inductive gene are reported during lateral root formation (Moreno-Risueno, 2010).

We established AUX/IAA19 promoter with improved luciferase system which report proximal to its promoter activity in temporal resolution. Exogenous auxin activate AUX/IAA19 promoter transient. This transient activation mechanism presumably caused by the time difference between reduction of AUX/IAA protein concentration and the velocity of de-novo synthesis of AUX/IAA protein family. Repetitive application activates AUX/IAA19 promoter, however, the kinetics of activation has been changed after first auxin application. This phenomenon suggests the identity of the tissue has been changed during auxin response, i.e. tissue memorize the experience of the history of auxin response, named “remodeling of auxin response”. AUX/IAA dominant mutation placed in domain II of Tir1-IAA binding pocket, results in gain-of-function phenotype. Such a dominant mutant, aux/iaa19/msg2 shows a defect of lateral root formation and remodeling of auxin response.

Poster presentation will discuss about the nature of transient activation of early auxin inducible gene and its role for organ formation.

Keywords: auxin, feedback regulation, luciferase imaging, AUX/IAA, Tir1/AFB, ARF
Poster/Talk #385. A meso-scale ABA interactome reveals a dynamic signaling landscape in Arabidopsis (Submission 99)
Shelley Lumba1, Shigeo Toh1, Louis-Francois Handfield1, Ji-Young Youn1, Sean R. Cutler2, Rajagopal Subramaniam3, Nicholas Provert1, Alan Moses1, Darrell Desveaux1, Peter McCourt1
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Abscisic acid (ABA) mediates an assortment of responses to various abiotic and biotic stresses in plants. A linear core pathway has been elucidated for transducing the ABA signal. However, the complexity of ABA-dependent gene expression suggests ABA functions through an intricate network. Here, using systems biology approaches that focused on genes transcriptionally regulated by ABA, we defined an ABA signaling network of over 500 interactions among 138 proteins. This map greatly expanded ABA core signaling but was still manageable for systematic analysis. For example, functional analysis was used to identify an ABA module centered around two SNF-like kinases. We also used co-expression analysis of interacting partners within the network to uncover dynamic sub-network structures in response to different abiotic stresses. This comprehensive ABA resource allows for application of approaches to understanding ABA functions in higher plants.

Keywords: Plant hormone signaling, ABA interactome, Systems biology, Protein interaction networks, Functional genomics, Targeted interactome, Abiotic stress

Poster #386. Ribosome footprinting and genetic analysis unveil the role of a novel translational regulation module in the Arabidopsis ethylene signaling pathway (Submission 111)
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Translational regulation has long been recognized as a potential point to control critical cellular processes as well as the responses to internal and external stimuli. Only recently, however, have we started to identify the molecular machinery responsible for such type of regulation and the corresponding gene targets. Using a genetic approach, we have identified new Arabidopsis weak ethylene insensitive mutants that also display defects in translation, suggesting the existence of a previously unknown molecular module involved in ethylene-mediated translation regulation of components of this signaling pathway. Our data indicate that this new level of regulation utilizes the nonsense-mediated mRNA decay (NMD) complex represented in our screen by mutations in the UPF1, UPF2 and UPF3 Arabidopsis genes. To explore this link in detail, we implemented the ribosome footprinting technology for Arabidopsis and examined the effects of short exposure to the hormone on the Arabidopsis transcriptome. Interestingly, several of the genes affected by ethylene at the translational level possess long 3’UTRs, a structural feature potentially involved in the regulation of translation. After the in vivo validation of the footprinting results, we have found that one of the 3’UTR investigated is sufficient to confer ethylene-mediated repression of translation to a reporter gene such as GFP, and that this 3’UTR is required for the proper plant responses to ethylene. We have also found that the translational regulation conferred by this 3’UTR is dependent of EIN2, key player in the ethylene signaling cascade, independent of EIN3 EIL1, master transcriptional regulators of the pathway, and unlikely to be mediated by miRNAs. The footprinting experiments also showed that this translation regulation by ethylene requires functional UPF2 and that mutations in upf2 result in a relative increase of ribosomal load in the 5’ upstream open-reading frames (uORFs) of target genes with respect to the downstream genic ORFs. Taken together, our results uncovered a novel mechanism for gene-specific translational regulation in response to plant hormones in Arabidopsis and showed that this regulation is UPF-dependent. They also imply that the NMD-triggered repression of translation can be uncoupled from the mRNA degradation processes and strongly suggest that a specific protein-mRNA interaction is responsible for the observed effects of ethylene on translation.

Keywords: Ethylene signaling, Ribosome footprinting, Gene-specific translation regulation

Poster/Talk #387. Recapitulation of the auxin response pathway in yeast (Submission 127)
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Auxin influences nearly every aspect of plant growth and development through a relatively simple pathway that pivots on the relief of transcriptional repression. Auxin triggers degradation of Aux/IAA repressors, thereby activating ARF transcription factors and triggering a global change in gene expression. The large size of the ARF and Aux/IAA gene families suggest that local differences in Aux/IAA-ARF composition could contribute to distinct signaling dynamics. However, characterization of individual Aux/IAA-ARF auxin response modules has been confounded by the ubiquity of auxin in plants, feedback, interactions with other auxin response components and interference from other signaling pathways. Here, we recapitulated the entire Arabidopsis thaliana forward auxin signal transduction pathway in Saccharomyces cerevisiae to assess if the composition of different modules was sufficient to produce distinct dynamic behaviors. Sensitivity analysis guided by a small mathematical model revealed the centrality of Aux/IAA co-repressors in driving response dynamics. In addition, we found that when multiple Aux/IAAs were co-expressed, one Aux/IAA could dominate the transcriptional dynamics. Our work provides a new method for dissecting auxin response dynamics and demonstrates the key role Aux/IAAs play in tuning the auxin response.

Keywords: synthetic biology, signaling dynamics, auxin

Poster/Talk #388. Can abscisic acid bind to and directly regulate ion channels? (Submission 155)
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1KAUST, Saudi Arabia
The plant hormone, abscisic acid (ABA) has important roles at different stages of plant development and is key to eliciting and/or modulating physiological responses to both abiotic and biotic stresses. To date, there is no evidence demonstrating direct interaction between ABA and ion channels despite the fact that ABA has been reported to affect ion channels in stomata. Here, we examine the effects of ABA on the outward K+-channel of Vicia faba guard cells using the patch-clamp technique in excised outside-out configurations. Membrane depolarization to values positive of the Nernst potential for K+ (EK) activated predominantly a 21 pS channel (111 mM K+ in : 11 mM K+ out). Addition of 20 - 100 mM of physiological active (+)-cis,trans-ABA stereoisomer into the bath medium increased K+-channel activity resulting in sustained opening times while the inactive trans,trans-ABA at similar concentrations had no effect. Importantly, this ABA effect on the K+-channel was reversed after ABA washout. These electrophysiology data suggest a membrane-delimited effect of ABA. We therefore predict that this ABA-modulated conductance may be due to the direct binding of ABA to the guard cell outward rectifying K+-channel (GORK) that may harbor a putative ABA-binding site at the cytosolic region of the molecule. Based on the alignment of conserved residues of known ABA-START receptors family protein, we have identified a candidate ABA-binding site. We further tested this site in an Arabidopsis GORK homolog (AT5G37500) by constructing a 3D homology model from which we have estimated the location of the ABA and the cyclic nucleotide-binding domains. We have also modeled the docking of (+)-cis-trans-ABA to the putative ABA-binding domain in GORK. The predicted region of binding (Gly313 - Leu731) was cloned and expressed in E. coli and the recombinant protein affinity purified. We are currently investigating the in vitro binding properties of ABA at the predicted ABA-binding site of the recombinant protein. Further work includes site-directed mutagenesis of key residues at the ABA-binding site and ex vivo studies involving the heterologous expression of the full-length GORK in mammalian cells followed by electrophysiological investigations.

Keywords: Abscisic acid (ABA), ABA binding site, Guard cell outward rectifying potassium channel (GORK), Homology modeling

Poster #389. Strigolactone modulation of systemic cytokinin homeostasis (Submission 178)
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Cytokinins (CKs) are both local and systemic signals that migrate through phloem and xylem. Zeatin CKs predominate in xylem sap whereas isopentenyl adenine (IP) forms are most abundant in phloem, but we do not fully understand the regulation of CK pool sizes and transport. Other signalling molecules impact on CK status: in particular strigolactone (SL) mutants have greatly depleted upward movement of xylem CKs, and auxin can
regulate expression of CK biosynthesis genes such as isopentenyl transferase (IPT), and the CYP735A enzymes that convert CKs to zeatin forms. Reporter gene expression patterns are different for each family member suggesting tight spatial regulation, yet single gene knockouts have few phenotypes. This strongly suggests that CK mobility enables systemic complementation of local defects. Here we attempted to shed light on some of the control points by testing three hypotheses: first, that some shoot-synthesised CKs recirculate via xylem; second, that compensatory CK movement will occur in grafts with CK-deficient (CK-) shoot or root; and third, that SL-mutations that suppress xylem CK may impact on normal CK homeostasis loops, resulting in attenuated phenotypic and hormonal complementation. Wild-type Arabidopsis shoots retained normal phenotypes on all grafted rootstock genotypes including doubly deficient CK- SL- (ipt3,5,7 max4 or cyp735a1,a2 max4) lines. Likewise wild-type rootstocks were able to rescue shoot phenotypes of all CK- and SL- biosynthetic mutants, and displayed elevated xylem CK content indicative of compensatory systemic CK transport. Extreme dwarf and branching CK- SL- shoots were rescued in shoot size if grafted to SL- roots. In contrast, SL- shoots of reciprocal grafts to CK- SL- roots unexpectedly displayed a dwarf phenotype. Both these results suggest a role for root-sourced CKs in regulating shoot growth, and we discuss how SL defects might perturb the normal systemic CK homeostasis mechanisms.

Keywords: cytokinin, strigolactone, xylem, phloem, homeostasis, feedback

Poster/Talk #390. SAUR proteins inhibit PP2C.D family phosphatases to control plant cell expansion (Submission 199)
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The plant hormone auxin controls numerous aspects of growth and development by regulating the expression of hundreds of genes. SMALL AUXIN UP RNA (SAUR) genes comprise the largest family of auxin responsive genes, but their function is unknown. We have recently demonstrated that members of the closely related SAUR19 subgroup of SAUR proteins (SAUR19-24) localize to the plasma membrane and promote cell expansion. These proteins are highly unstable in Arabidopsis. However, the addition of an N-terminal GFP or epitope tag dramatically increases their stability, enabling a gain-of-function genetic approach to study SAUR protein function. Arabidopsis plants expressing stabilized SAUR19-24 fusion proteins exhibit several phenotypes indicative of increased and/or unregulated cell expansion, including increased hypocotyl and leaf size, defective apical hook maintenance, and altered tropic responses. Reciprocally, plants expressing an artificial microRNA targeting multiple members of the SAUR19 family members exhibit short hypocotyls and reduced leaf size. We find that SAUR proteins both interact with, and inhibit the enzymatic activity of, members of the D-clade of type 2C protein phosphatases. Consistent with SAUR and PP2C.D proteins acting in an antagonistic fashion, we find that pp2c.d family mutants exhibit increased growth phenotypes similar to those conferred by SAUR19 overexpression. Reciprocally, PP2C.D overexpression confers reduced plant growth phenotypes by inhibiting cell expansion. Our findings reveal that SAUR and PP2C.D proteins control cell expansion by regulating the phosphorylation status and activity of plasma membrane (PM) H+ ATPases. The C-terminal autoinhibitory domain of PM H+ ATPases is hyperphosphorylated and hypophosphorylated in plants overexpressing SAUR19 and PP2C.D1, respectively. These changes in PM H+-ATPase phosphorylation status result in reciprocal changes in PM H+-ATPase activity. Furthermore, the increased growth phenotypes conferred by SAUR19 overexpression require normal PM H+ ATPase function. Our findings provide a molecular framework for elucidating auxin-mediated control of plant cell expansion and provide key molecular support for the decades old acid growth theory of plant cell expansion.

Keywords: auxin, SAUR, cell expansion, PP2C phosphatases, plasma membrane H+ ATPase

Poster #391. Functional Genomics of Cytokinin Response Factors: Analysis of a Diverse Sub-Group of AP2/ERF Transcription Factors (Submission 202)
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Cytokinin Response Factors (CRFs) are a small group of AP2/ERF transcription factor proteins in plants that
include 12 members in Arabidopsis. Founding members of this group were originally identified in Arabidopsis as being strongly induced by the plant hormone cytokinin and orthologs of these genes in other species like tomato show similar strong cytokinin regulation. However, additional analysis has revealed that not every CRF member is transcriptionally regulated by this hormone and the question remains as to what regulates these other members. Phylogenetic analysis of hundreds of CRF sequences from across Angiosperm species places almost every CRF into one of 5 distinct clades of related sequences. Interestingly, there are no Arabidopsis CRFs in one of these well conserved clades. We have used a comparative genomic approach based on this phylogenetic grouping, transcriptional analyses, and direct experimentation from which it appears that each CRF clade may have a unique functional role in either hormone and/or stress response. Here we will present findings of several CRFs (CRF2, CRF4, CRF6, and CRF9) that belong to distinct CRF clades along with one CRF (CRF7) that does not clearly fit into any group. Results include examinations of mutant, overexpression, and reporter gene lines under standard, hormone, and stress treatment conditions; in addition to changes to transcription levels. Together this shows the initial analysis of a functionally diverse group of CRF genes involved in a range of cytokinin related responses.

Keywords: Cytokinin, CRF, Transcription Factor

Poster #392. ABI5-binding proteins (AFPs) interact with histone deacetylase complex subunits and repress transcription (Submission 217)
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Abscisic acid signaling involves interactions among positive regulators of gene expression, such as the ABA-INSENSITIVE(ABI)5/ABA Response Element (ABRE)-binding factor (ABF/AREB) clade of bZIP transcription factors, and negative regulators of their stability or action. A small family of ABI5 interacting proteins, designated AFPs, was identified by a yeast two-hybrid screen using ABI5 as "bait." Physiological and genetic characterization showed that most of the AFPs were induced by ABA or dehydrating stresses, but at different stages in seedling development, and most acted as repressors of ABA and stress response. The AFPs have three highly conserved domains, including an ethylene-responsive element binding factor-associated Amphiphilic Repression (EAR) domain, a nuclear localization signal, and a C-terminal domain that interacts with ABI5 and related bZIPs. The mechanism of action for the AFPs has remained controversial, with proposed functions ranging from promoting ABI5 degradation to TOPLESS-dependent chromatin modification, analogous to the function of the related NINJA protein in jasmonate signaling. We have directly tested whether the AFPs act as transcriptional repressors in a heterologous system, using reporter genes in yeast regulated by either constitutive yeast promoters or ABF-inducible ABRE-regulated promoters. Our studies show that, although all four AFPs share the EAR domain implicated in interaction with TPL, only three of them have significant intrinsic repressor activity. Furthermore, the EAR domain is not necessary for repressing either the constitutive promoter or ABF-activation of ABRE-containing promoters in yeast. The latter effect depends on the ability to interact with the bZIPs. In addition, some of the AFPs interact with subunits of the histone deacetylase complex. Consistent with this, over-expression of tagged AFPs lacking the EAR domain is sufficient to confer resistance to ABA inhibition of germination. Strikingly, strong over-expression of YFP-AFP2 confers seed maturation defects including a "green seed" phenotype and at least a 300-fold increase in resistance to ABA at germination.

Keywords: abscisic acid, ABI5, AFP, histone deacetylase

Poster/Talk #393. TO GROW OR NOT TO GROW: HORMONAL REGULATION OF FITNESS TRADE-OFFS IN ARABIDOPSIS (Submission 219)
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Plants, like all other organisms, need to assess and adapt to constant environmental changes throughout their life cycle. Responding to biotic stress is a very energy-costly process, and therefore requires tightly controlled mechanisms to ensure activation of appropriate levels of defense responses. The molecular mechanisms that control metabolic reprogramming from cellular growth to activation of defense responses (growth-to-defense
transition) are not well understood. The role of plant hormones in regulating these processes has been suggested, but only a few examples of hormonal interplay have been identified and characterized. With advancements in high throughput molecular techniques and computation data analysis, tools are now available that allow us to begin this type of investigation to gain a better understanding of how plant hormones shape the outcome of defense activation and its consequences to plant growth. We employed transcriptomics and targeted metabolomics profiling to monitor global gene expression and the levels of all major plant hormones (abscisic acid, auxin, brassinosteroids, cytokinin, gibberellic acid, jasmonic acid and salicylic acid). A hormonal interplay between two plant hormones, salicylic acid and cytokinins, was identified that may play a key regulatory role in the control of the growth-to-defense transition. Results from this project will help understand how plant hormones regulate plant metabolic processes linked to growth and defense to pathogens and may explain fitness costs associated with constitutive defense activation.

Keywords: cytokinin, salicylic acid, metabolomics, transcriptomics, plant growth, plant defense

Poster #394. Small-molecule screening in Arabidopsis and yeast uncovers agonists for the strigolactone receptor HTL/KAI2 that are bioactive in Arabidopsis and Striga hermonthica (Submission 257)
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Strigolactones (SLs) are small-molecule plant hormones that regulate development. In addition to their endogenous hormonal roles, SLs are secreted into the soil where they act as signaling molecules that stimulate hyphal branching in symbiotic mycorrhizal fungi. However, parasitic plants of the genera Striga and Orobanche have evolved to hijack this symbiotic interaction by using secreted SLs as a cue for germination, and parasitism. Striga causes billions of dollars of crop damage in the developing world per year, and thus understanding SL signaling is of enormous interest.

Because Striga is not a genetically tractable organism, efforts to understand SL signaling have been made in a variety of model plants, generating the following basic model: In Arabidopsis, a small family of a/b hydrolases serve as receptors for SLs. In Arabidopsis, the paralog that is thought to be most important for perceiving SLs within the context of germination and early seedling growth is HTL/KAI2, whereas the paralog that is more important in other areas of development such as in the suppression of branching is known as AtD14. The AtD14 receptor has been shown to physically interact with MAX2, an F-BOX protein and essential SL signaling component, in a SL dependent manner. Recent reports have indicated that a variety of other proteins may also interact with these receptors. With respect to HTL/KAI2, exogenous SL application is known to ablate the nuclear localization of the E3 ubiquitin ligase COP1, allowing the accumulation of transcription factors including HY5, and thus causing changes in the transcription of downstream genes.

These molecular details of SL signaling allowed the design of a series of chemical screens intended to identify HTL/KAI2 agonists. Certain screening leads were shown to alter the nuclear localization of a GUS-COP1 fusion protein, and were also shown biochemically to interact directly with HTL/KAI2. Additionally, these lead compounds were able to stimulate the germination of Striga hermonthica. This suggests that these compounds are able to mimic SLs and that they act directly on the receptor. One suggested approach to reducing the intensity of Striga infestations is to stimulate their seeds’ germination in the absence of a host. These compounds could serve as the basis for the development of more potent “suicide germination” compounds. Additionally these mimics could be used as molecular probes to further characterize SL signaling.

Keywords: Hormone signaling, Chemical biology, Germination, Biotechnology

Poster #395. Characterization of mutants with enhanced resistance to IBA (Submission 261)
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Plant peroxisomes are small dynamic organelles that mediate numerous processes in primary and secondary metabolism crucial for growth and development. Peroxisome functions include inactivation of hydrogen peroxide, fatty acid b-oxidation, the glyoxylate cycle, photorespiration and production of the phytohormones indole-3-acetic acid (IAA) and jasmonic acid (JA). Plant peroxisomes exclusively carry out fatty acid b-oxidation in which oil
Abscisic acid (ABA) plays an important role in the control of growth, development and stress responses in plants. Numerous growth and developmental processes including lateral root induction and inhibition of primary root elongation. IBA has similar effects as IAA on root development, but is superior at inducing lateral roots. A screen identified enzymes functioning in IAA to IAA conversion, including proteins involved in peroxisomal biogenesis and import. These IBA response mutants (ibr) are resistant to IBA but remain sensitive to IAA. To further explore mechanisms that directly influence IBA to IAA conversion or generally affect peroxisome functions, ibr-1 and ibr-1 were mutagenized for a screen seeking mutants with enhanced IBA resistance. Characterization of enhancer mutants will further reveal the individual roles of IBR3 and IBR1 while identifying additional players involved in IBA metabolism. Here we describe the initial characterization of our mutants. Mutants isolated from the ibr-1 background are IBA resistant in roots and hypocotyls. The ibr-1 screen yielded mutants with varying IBA resistant phenotypes that can be separated in roots and hypocotyls. Two mutants have been characterized in detail: Z377ibr3-1 and Z377 enhancer mutants both have IBA-response phenotypes but germinate normally and are not sucrose dependent, suggesting a specificity to IBA responses. The causative mutation of Z353 was identified as ACX3, a fatty acid b-oxidation enzyme. Preliminary characterization to determine IBA resistance of acx3ibr3 and z353ibr3-1 is presented along with effects on fatty acid b-oxidation. ACX3 and IBR3 are hypothesized to function in the same step of their respective pathways. Characterization of acx3ibr3 will provide insight into the extent of interaction between these two pathways.

Keywords: Indole-3-butryic acid, IBA, Indole-3-acetic acid, IAA

Poster #396. Functional Analysis of Arabidopsis thaliana OVATE FAMILY PROTEINS (Submission 299)  
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Ovate Family Proteins (OFPs) belong to a plant-specific family of regulatory proteins that act as transcriptional repressors, and are encoded by a family of 18 genes in Arabidopsis. Among all OFP family members, OFP1 was shown to regulate cell elongation, partly by suppressing the expression of GA20ox1. OFP4 was shown to interact with KNAT7, a TALE homeodomain protein (HD), to regulate secondary cell wall biosynthesis. To identify novel OFP4 interaction partners, we used yeast two-hybrid and bimolecular fluorescence complementation assays and NAP1 (Nucleosome Assembly Protein 1) family proteins, NAP1;1 and NAP1;2, were shown to interact with OFP4 in vitro and in vivo. Both NAP1;1-YFP and NAP1;2-YFP fusion proteins were localized abundantly in the cytoplasm, associated with the ER. Further work is underway to confirm and evaluate the biological significance of the OFP4-NAP1 interaction. Ectopic expression of both OFP2 and OFP5 caused pleiotropic developmental defects similar to OFP1 and OFP4 over-expression phenotypes, including dwarfism, round and curled leaves, delayed development, and reduced fertility. Furthermore, the hypocotyls of OFP2 and OFP5 over-expression plants showed cell swelling and disordered microtubule phenotypes. In dark conditions, both mutants exhibited de-etiolated phenotypes, resembling brassinosteroid (BR) deficient mutants. Exogenous BR treatment partially rescued the phenotypes of plants over-expressing both OFP2 and OFP5. Meanwhile, the expression of DET2 (De-etiolated2), a BR biosynthetic gene, was decreased significantly in the OFP2 and OFP5 over-expression plants. We hypothesize that OFP2 and OFP5 may function redundantly with OFP1 and OFP4, to negatively regulate BR levels in plants. Taken together, our study provides new insights into OFPs functions involved in maintaining BR homeostasis in Arabidopsis.

Keywords: Ovate Family Proteins, protein-protein interactions, Nucleosome Assembly Protein1, Brassinosteroid, DET2 (De-etiolated2)

Poster #397. Characterization of NCED and CYP707A gene families encoding ABA metabolic enzymes of Arabidopsis (Submission 303)  
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Abscisic acid (ABA) plays an important role in the control of growth, development and stress responses in plants.

Keywords: Ovate Family Proteins, protein-protein interactions, Nucleosome Assembly Protein1, Brassinosteroid, DET2 (De-etiolated2)
ABA level is regulated through regulation of its biosynthesis and catabolism. Nine-cis epoxycarotenoid dioxygenase (NCED) is the rate-limiting enzyme in the ABA biosynthesis. The NCED cleaves 9-cis epoxycarotenoids into xanthoxin. ABA 8'-hydroxylase (CYP707A) is a regulatory enzyme in the ABA catabolism and oxidizes ABA into 8'-hydroxy ABA and 9'-hydroxy ABA. The Arabidopsis genome has 5 NCED genes and 4 CYP707A genes which are differently expressed spatially and temporally. However, little is known about post-transcriptional or post-translational regulations of these genes/enzymes. We are interested in elucidating how these genes/enzymes are regulated and if these show differential regulations among the family members. We have expressed these genes using the same promoter (Cauliflower Mosaic Virus 35S promoter) in plants to compare the effect of overexpression of these genes. We will present phenotypic characterization of these transgenic lines.

Keywords: ABA metabolism, CYP707A, NCED, Abscisic acid, ABA

Poster #398. Nuclear Factor Y-mediated SOC1 expression orchestrates flowering responses of Arabidopsis (Submission 325)
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Nuclear factor Y (NF-Y) complexes consisting of three subunits are conserved combinatorial transcription factors in eukaryotes. Despite a significant increase in the number of NF-Y subunits in the plant lineage, the precise function of complete NF-Y complexes in plants is unknown. Here we report the discovery of a complete NF-Y complex that regulates epigenetic control of flowering time through integrating environmental and developmental signals in Arabidopsis. NF-Y subunits interact with CONSTANS in the photoperiod pathway and DELLAs in the gibberellin pathway to directly regulate the transcription of SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (SOC1), a major floral pathway integrator. NF-Y binds to a unique cis-element in the SOC1 promoter and modulates H3K27me3 dynamics at SOC1 locus. Our findings establish NF-Y complexes as critical regulators that orchestrate plant responses to environmental and intrinsic signals to mediate epigenetic control of key developmental transitions.

Keywords: flowering time, nuclear factor Y, CONSTANS, gibberellins, SOC1

Poster #399. Auxin or high temperature controls the symptoms of necrotic spotted lesion 2 (nsl2) (Submission 326)
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The plant phytohormone auxin regulates diverse processes during cellular and developmental response. Auxin function involved in plant immunity response is also implicated, endogenous indole-3-acetic acid (IAA) level increases upon pathogen infection (O'Donnell et al., 2003); enhancement of antibacterial resistance is connected with suppression of auxin signaling (Navarro et al., 2000). Previous study of constitutive cell death mutant nsl2 (originally reported as the constitutively activated cell death 1 (cad1)), showed localized cell death on development of leaves, high accumulation of salicylic acid accompanied with elevated expression of pathogenesis-related genes (Yamamuro et al., 2005; Tsutsui et al., 2008). Besides of such a plant immunity response, nsl2 displays severe dwarfism, low fertility, ion-leakage and leaves chlorosis which implicate aberrant hormonal responses.

Among tested phytohormones, an application of exogenous IAA partially restored the phenotypes of nsl2, for example plant size, chlorosis extent, and leaves ion leakage was decreased by 40% compared to nsl2 control. To demonstrate the effects of endogenous auxin on nsl2 phenotype, high temperature treatment was tested which activates the biosynthesis of endogenous auxin (Gray et al., 1998; Franklin et al., 2011). Consistent with the results of exogenously applied IAA, nsl2 cell death phenomenon was suppressed by high temperature treatment, suggesting elevated endogenous auxin recovered nsl2 cell death phenotype. Partial recovery nsl2 cell death phenotype with elevated endogenous IAA or exogenous IAA potentially suggests NLS2 is participated in biosynthesis of IAA or auxin-signaling. On the other hand nsl2 mutant showed more elongated hypocotyl length.
and arrested primary root growth under high temperature, indicating nsl2 mutant is hypersensitive to high temperature and auxin response. Poster presentation will discuss about the role of auxin during tissue maintenance for photosynthetic organ and organogenesis. Furthermore novel auxin functions are clarified by utilizing constitutive cell death mutant nsl2, cross talk among cell death, auxin response and high temperature response are performed as well.

Keywords: auxin, high temperature, NSL2, cell death

Poster #400. Identification of gravitropism-related PIN3 polarity regulators in Arabidopsis (Submission 334)
Hana Rakusova1, Jiri Friml2
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Tropisms are processes for plants to adapt to environmental changes such as light (phototropism) or gravity (gravitropism). Gravitropism is a growth reaction orienting plant's development parallel to earth's gravitational field. Roots grow with the gravity vector, whereas shoots grow against it. Gravity-induced redistribution of the phytohormone auxin mediates tropic responses both in roots as in shoots. In both instances PIN3 auxin transporter is important for this process. PIN3 re-localizes in response to gravistimulus to redirect the auxin flow and achieve asymmetric auxin distribution. Regulations of PIN3 subcellular localization and subsequently PIN3-dependent auxin transport are thus crucial process for tropic bending.

We designed a forward genetic screen to identify mutants defective in PIN3 re-polarization after gravity-stimulation. To circumvent the problem of phenotype scoring at the cellular level into easy-to-visualize morphological output manifested by the hypocotyl bending response. The screen is based on the gravitropic hypocotyl growth in the transgenic PIN3::PIN3-GFP background. The screen has been designed considering the hypothesis that the EMS-induced mutations in putative regulators of PIN3 re-polarization will lead to a defect in gravity-mediated PIN3 re-location to trigger no auxin accumulation in the bottom side of hypocotyl and to prevent bending. In the light of our promising results, this forward genetic screen will identify novel PIN3 polarity components and will provide insights into the mechanism of PIN3 cell polarity establishment, maintenance and its dynamic regulations as well as mechanism of gravity signal transduction in plants.

Keywords: gravitropism, auxin, PIN3

Poster #401. Identification of the PP2C HAI1 as an Interactor of ABA2, an ABA Biosynthetic Enzyme (Submission 335)
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Abscisic acid (ABA) is a plant hormone that plays a regulatory role in the water stress response, as well as the maintenance of seed dormancy and the mitigation of other abiotic stresses (1). ABA2 is an ABA biosynthetic enzyme, which converts xanthoxin to abscisic aldehyde (1). Its expression is not coordinated with the expression of other ABA biosynthesis genes, as well as with stress (2). Perhaps this points to a unique function of ABA2, one that lies beyond the realm of ABA biosynthesis and requires its expression to remain constant. In order to elucidate potentially novel roles of ABA2, we analyzed interaction partners of this enzyme by conducting yeast-two hybrid (Y2H) assays using a full-length cDNA collection for ABA inducible genes (3). Using this assay, we found that ABA2 interacts with itself (4), which is consistent with the finding that ABA2 functions as a multimeric complex (5). HAI1, a Protein Phosphatase Type 2 C (PP2C), was also found to interact with ABA2, in addition to other members of this family, including ABA-hypersensitive germination3 (AHG3) (4) and hypersensitive to ABA1 (HAB1). These proteins are members of the core ABA signaling complex and act as negative regulators of ABA-induced gene expression (6). We are currently examining if ABA2 and PP2Cs interact in planta by BiFC. We will discuss the possible interaction between ABA biosynthesis and signaling at a protein level.

3) Lumba S et al. 2014. A Mesoscale Abscisic Acid Hormone Interactome Reveals a Dynamic Signaling Landscape in

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Poster #402. Indole-3-acetic acid and phenylacetic acid are two types of auxins in Arabidopsis with distinct transport characteristics (Submission 358)
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Auxin plays a crucial role in various aspects of plant growth and development. Indole-3-acetic acid (IAA) is the most studied auxin that possesses polar transport property. Phenylacetic acid (PAA) has also been recognized as a natural auxin, but its role in plant growth and development remains unclear. In this study, PAA biosynthesis, inactivation, transport, and signal transduction in Arabidopsis are investigated. Genetic and biochemical analyses demonstrate that the YUCCA family plays an important role in both IAA and PAA biosynthesis, and the GH3 family inactivates these two auxins through conjugation with amino acids. However, unlike IAA, PAA does not recover root gravitropism of auxin-deficient mutants, indicating that IAA and PAA have distinct transport characteristics. IAA and PAA can regulate the same set of auxin responsive genes but also various genes independently, suggesting that these two auxins have overlapping but distinct regulatory roles. These results demonstrate that PAA probably functions as an auxin in various plant species, thus providing new insights into the regulation of plant growth and development by multiple auxins.

Keywords: plant hormone, auxin, biosynthesis, metabolism, polar transport, signal transduction

Poster #403. An ABC transporter mediates ethylene signaling under HY5-regulation (Submission 384)
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Plant growth and development are affected by exogenous cues such as light, and endogenous cues such as hormone. The combined regulation on developmental processes enables the plant to adapt to their ever-changing environmental conditions. Thus the light-regulated processes often require specific hormonal signaling, and hormone signaling is affected by light conditions. Here we report an ABC protein ABC9 which seems to be important for such cross talk. ABC9 transcripts were induced by light, and the induction of ABC9 was abolished in hy5-1 mutants, indicating that the induction of ABC9 gene is dependent on function of HY5, a key regulator of light signaling in Arabidopsis. In line with this result, ABC9 promoter region contains G-box and ACE, which are binding sites of the HY5 transcription factor. ABC9 does not have any trans-membrane domain, and thus is predicted to be a soluble ABC protein. However, in plant cells the fluorescence of GFP-tagged ABC9 protein was localized at the endoplasmic reticulum (ER) membrane. ABC9 did not dissociate from the membrane by treatment with a 100 mM of KCl, but only when treated with Na2CO3, it was detected in the soluble fraction, suggesting a tight association with the membrane. Based on these results, we hypothesized that ABC9 mediates ethylene signaling on the ER membrane under HY5-regulation. Analyses of the only available knockout mutant abc9-1 revealed that the mutant could not show triple responses to ethylene in medium containing 1-aminocyclopropane-1-carboxylic acid (ACC). The anthocyanin content of abc9-1 was much increased than in wild type, indicating suppressed ethylene signaling. In the transcriptomic analysis of the abc9-1, many ethylene signaling genes were differentially expressed, supporting the involvement of ABC9 in ethylene signaling. We are generating CRISPR/Cas9-based ABC9 knockout mutants to test whether the phenotypes of abc9-1 are indeed due to the

Keywords: ABA2, HAI1, PP2C, ABA biosynthesis
absence of ABC9, and to test our hypothesis.

Keywords: ABC transporter, Ethylene, HY5

Poster #404. Root-derived active cytokinins regulate shoot growth in Arabidopsis (Submission 397)
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In order to coordinate growth and development of distant organs like shoot and root in response to environmental changes, organ-to-organ communications are required. A number of metabolites and hormones translocated via xylem and phloem play a role in the communication as signaling molecules. Cytokinins, a group of phytohormones, have been proposed to act as such molecules in response to various environmental cues such as light, nitrogen and carbon nutritional status. Because cytokinin ribosides, precursors of active cytokinins, are the major form of cytokinin in xylem and phloem, current understand is that cytokinin ribosides are the form of transportation and they are activated by cytokinin activating LONELY GUY (LOG) enzyme-dependent pathway at the site of action [1]. Therefore no attention has been paid to the role of active cytokinins as a long-distance signal, though a small amount of them also exist in xylem and phloem.

To test whether active cytokinins are translocated from root to shoot and play a signaling role, we conducted grafting between root of wild-type and shoot of log septuple mutant (logS) which cannot generate active cytokinins by LOG-dependent pathway [2]. We found that the content of active cytokinins was recovered to wild type level in logS shoot grafted onto wild type root. Consistently, expressions of many cytokinin-responsive type-A ARABIDOPSIS RESPONSE REGULATOR genes were restored in the shoot of grafted plants. However when we analyzed the shoot phenotypes (i.e. leaf size, inflorescence stem thickness and plastochron etc.) of grafted plants, we found that not all phenotypes were recovered. In addition, expressions of several cytokinin-responsive type-A ARABIDOPSIS RESPONSE REGULATOR genes were not recovered. These results suggest that active cytokinins are indeed transported from root to shoot and that the root-borne active cytokinins play a role in some aspects of shoot growth regulation. In our presentation, we will discuss about the physiological role of root-borne active cytokinins in shoot growth in comparison with that of root-borne cytokinin ribosides and shoot-borne active cytokinins.


Keywords: cytokinin, long-distance signal, transport

Poster #405. GAF1, A DELLA INTERACTING PROTEIN, REGULATES GIBBERELLIN HOMEOSTASIS AND SIGNALING (Submission 408)
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Gibberellins (GAs) are important phytohormone for plant growth and development. DELLA proteins are members of the plant-specific GRAS protein family and act as repressors of the GA signaling pathway. DELLA proteins are rapidly degraded in the presence of GA. Recently it have been reported that DELLA proteins inhibit transcription factors in several signaling through protein-protein interaction. Mechanism of GA-dependent transcription has been explained by the titration of transcriptional activators with DELLA and their release through degradation of DELLA in response to GA. However, the effect of GA on genome-wide expression is predominantly repression, suggesting the existence of unknown mechanisms. We have identified a DELLA interacting protein, named GAF1, using modified Y2H screening. GAF1 is a novel transcriptional factor that binds to DNA in a sequence-specific manner. The Arabidopsis plants that overexpressed GAF1 exhibited the phenotypes of early flowering with larger leaves. In contrast, gaf1 gaf2 mutant exhibit semi-dwarf and late flowering phenotypes. GAF1 binds to genes under control of GA feedback regulation. Furthermore, we found that GAF1 interacts with corepressor TOPLESS Related (TPR). Molecular analysis suggests that GAF1 interacts with DELLA and TPR protein in nuclei. DELLA and TPR act as a coactivator and a corepressor of GAF1, respectively. GA converts GAF1 complex from transcriptional activator to repressor via degradation of DELLA. The DELLA turns on or off two sets of GA-
regulated genes by dual functions, namely titration and co-activation, providing a novel mechanism for integrative regulation of plant growth and GA homeostasis.

Keywords: gibberellin, transcription factor, DELLA protein, GA homeostasis

Poster #406. UGT74D1 catalyzes the glucosylation of 2-oxindole-3-acetic acid in Arabidopsis thaliana (Submission 422)
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Indole-3-acetic acid (IAA) is a naturally occurring auxin that plays a crucial role in many aspects of plant growth and development. The endogenous concentration of IAA is spatiotemporally regulated by biosynthesis, transport and inactivation in plants. Previous studies have shown that IAA is converted to 2-oxindole-3-acetic acid (OxIAA) and its glucoside, OxIAA-glucose (OxIAA-Glc) in Arabidopsis. The endogenous level of OxIAA-Glc is much higher than that of other IAA metabolites in Arabidopsis, suggesting that the OxIAA pathway is an important metabolic pathway for IAA homeostasis. However, no enzyme involved in the OxIAA pathway had been identified. In this work, we demonstrate that UGT74D1 catalyzes glucosylation of OxIAA in vitro and in vivo. We screened Arabidopsis UDP-glycosyltransferase (UGT) genes by using yeast expression system, and found that the yeast expressing UGT74D1 produces OxIAA-Glc in the presence of OxIAA. Further we confirmed that recombinant UGT74D1 enzyme catalyzed the conversion from OxIAA to OxIAA-Glc in vitro. To assess the physiological function of UGT74D1 in plant, we analyzed T-DNA insertion mutant of UGT74D1. Endogenous OxIAA-Glc level in ugt74d1 mutant decreased by 85% compared with that in wild type plant. On the other hand, OxIAA level in the mutant was 2.5 times higher than that of wild type. These results indicate that UGT74D1 plays a pivotal role in the production of OxIAA-Glc in Arabidopsis. We also reveal that overexpression of UGT74D1 resulted in root agravitropism as observed in that of GH3.6, IAA-amide synthetase. The supplement of IAA recovered gravitropism in UGT74D1 overexpressed plants, suggesting that root agravitropism was caused by the decrease of auxin level. We conclude that UGT74D1 catalyzes glucosylation of OxIAA and may contribute to cellular auxin homeostasis in Arabidopsis.

Keywords: auxin, glycosyltransferase, Arabidopsis

Poster #407. Hormonal cross-talk between brassinosteroids (BR) and abscisic acid (ABA) indicates an antagonistic role for BR in ABA responses and drought tolerance (Submission 423)
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Due to their sessile nature, plants are constantly exposed to a multitude of environmental stresses, most notably, drought stress. The sesquiterpenoid phytohormone abscisic acid (ABA) plays an essential role in triggering stomatal closure to prevent excessive water loss during drought conditions. Recently, emerging evidence suggest that other hormones, such as brassinosteroids (BRs), are also involved in mediating a range of abiotic stresses (Kagale et al., 2007) including drought tolerance. BR deficient and signaling mutants have been reported to be hypersensitive to ABA (Rodrigues et al., 2009), suggesting a possible cross-talk between these two phytohormones. BRs are sterol-type hormones that are involved in multiple processes of plant development and physiology. In the BR biosynthetic pathway, the rate-limiting step of conversion of castasterone (CS) to brassinolide (BL), the most bioactive BR, is exclusively catalyzed by the enzyme BR6OX2 (encoded by CYP85A2 gene). Subsequently, BL binds to its receptor to activate BR signal transduction. Therefore, plants lacking CYP85A2 is a great starting point to understand the cross-talk between these pathways. We have characterized ABA hypersensitivity of cyp85a2 mutant and investigated the mechanism of BR-mediated repression of ABA responses in Arabidopsis. cyp85a2 mutant phenotypically resembles BR biosynthetic mutants, exhibiting slightly dwarfed stature, rounded leaves, and increased lateral branches. It displayed ABA-hypersensitivity in germination assays and drought test. Genome-wide transcriptomic analysis indicated an up-regulation in ABA responsive genes, despite having no change in ABA concentration. Double mutant analysis with ABA biosynthetic and signaling mutants in cyp85a2 background indicated an antagonistic interaction of BR with ABA at the signaling
level. Our collective data demonstrate that BR can suppress ABA signalling independent of the ABA biosynthetic pathway.

Keywords: Hormonal cross-talk, Brassinosteroids (BR), Abscisic acid (ABA), Drought tolerance

**Poster #408. Development of new undergraduate research projects on plant hormones in relation to growth in Arabidopsis mutants and transformed plants** (Submission 446)

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Plant hormones are signaling molecules that control plant growth and development via their spatiotemporal concentration gradients. Understanding how different plant hormones interact to regulate plant growth and development is an important aspect of plant hormone biology. The first experiment focused on studying the effect of plant hormones such as indole acetic acid (IAA), zeatin (Z) and epi-brassinolide (EBR) on the spatial expression pattern of auxin in 6-day old DR5::GUS transformed Arabidopsis thaliana plants. The DR5::GUS construct used in this study, contains an auxin-response element that is sensitive to increasing endogenous auxin concentrations and allows us to visualize auxin distribution within the plant. Endogenous auxin was concentrated at root tips, cotyledons, lateral root primordia, apical meristem, and the leaf margins. EBR amplifies endogenous auxin expression in the roots and leaf vascular tissues, whereas Z reduced the auxin expression in the roots. The second experiment focused on investigating the effects of plant hormones, IAA and EBR on the root elongation rate in wild-type and the temperature-sensitive mutants of microtubule assembly (mor1-1) and cellulose synthesis (rsw1-1) of Arabidopsis thaliana. IAA (10-9 - 10-6 M) inhibited root growth but increased lateral root formation in both wild-type and mutants. EBR increased the root growth especially at low concentrations (e.g. 10-8 M) and induced curvature of the root tip. The roles of plant hormones in growth in Arabidopsis wild-type, mutants and transformed plants will be discussed. The results of this investigation have led to the development of new laboratory research projects on hormone biology in two undergraduate plant physiology courses.

Keywords: Indole acetic acid, Epi-brassinolide, Zeatin

**Poster #409. FAB1/PIKfyve establishes late endosomes/PVC/MBV identity in Arabidopsis thaliana** (Submission 447)

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Establishment of cell polarity is vital for all multicellular organisms including plants. In plants, auxin is known as a phytohormone which is a key molecule having a wide variety of physiological role encompassing growth, development and tropic response. Auxin moves particular direction by cell-to-cell polar transport mediated by auxin transporters, such as AUX1, PINs, and PGPs. Particularly, PIN family proteins are known to polarly distribute on particular domain of the plasma membrane. The polarity of PIN protein is established by the local recycling membrane trafficking pathway composed of early endosomes/TGN, late endosomes/PVC/MVB, and recycling endosomes. In eukaryotes, PtdIns 3, 5-kinase, Fab1/PIKfyve produce PtdIns (3, 5)P2 from PtdIns 3-P. PtdIns(3, 5)P2 has a multiple roles in maintenance of lysosome/vacuole morphology and acidification, membrane trafficking of proteins, autophagy and signaling mediation in response to various stresses. However, the factor and molecular mechanism of the establishment of PIN polarity is largely obscure. We have previously reported that conditional knockdown mutant shows various auxin phenotype, which is not only root growth inhibition and hyposensitivity to exogenous auxin, but also impairment of root gravitropism. Here, we found that novel factor for PIN polarity and for the establishment of late endosomes/PVC/MVB. This defect was caused by improper distribution of auxin in root epidermal and cortical cells when gravity direction was changed. We also found that knockdown of FAB1 inhibits gravity-induced PIN2 degradation at upper side of epidermal and cortical cells. FAB1 co-localized with SNX1 in late endosomes/PVC/MVB. Furthermore, we found that FAB1 was physically interacted with SNX1. The endosomal localization of SNX1, Ara7 but not SYP43 and VAMP727, indicating that FAB1 mediates the late endosome localization of SNX1 and Ara7. Taken together, our results indicate that FAB1 is a key molecule of the recycling and polarity determination of PIN2, FAB1 plays an essential role for rootward position of the polar
localization of PIN2 by establishing late endosomes/PVC/MVB identity in Arabidopsis.

Keywords: auxin, Fab1/PIKfyve PIN2, SNX1

Poster #410. Role of PP2C MAPK phosphatases in stress hormone production (Submission 458)
Alois Schweighofer1, Volodymyr Shubchynsky2, Kotryna Kvederaviciute1, Michael Stumpe1, Justinas Simulis1, Felix Mauch2, Irute Meskiene1
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Pathogens stimulate intracellular signaling pathways in plant cells that initiate defense responses, including activation of plant stress hormone production. Pathogen-triggered immunity (PTI) includes phosphorylation-based activation of mitogen-activated protein kinases (MAPKs), which is counteracted by protein phosphatases. In Arabidopsis the pathogen-induced MAPKs MPK3, 4 and 6 play a role in the regulation of stress hormone production. In Arabidopsis MAPK phosphatases regulate MAPK activities and can affect signaling responses. We have identified Arabidopsis PP2C-type protein phosphatases AP2Cs as MAPK phosphatases, which interact with and inactivate MPK3, 4, and 6. We found that these phosphatases dephosphorylate Arabidopsis MPK6 on phospho-T residue localized in the activation loop of the MAPK and thus inactivate its kinase activity. MAPK activation leads to reprogramming of plant cellular activities, including changes in plant stress hormone levels.
Thus, we studied the role of AP2C PP2C-type MAPK phosphatases in regulation of kinase activities, salicylic acid and ethylene production during PTI. We correlated kinetic profiles of pathogen-induced kinase activities in phosphatase knock out and overexpressing Arabidopsis plant lines with the production of stress hormones salicylic acid and ethylene. Our data show that overexpression of the phosphatase leads to strong suppression of kinase activities, whereas in the absence of the phosphatase kinase activities are enhanced. We observed changes in ethylene amounts produced in these plants after pathogen-induced stress in comparison to wild type plants. Taken together, this work advances current understanding on regulation of MAPK signaling in plants and highlights the regulatory role of PP2C type MAPK phosphatase in PTI.

Keywords: protein phosphatase, PP2C, salicylic acid, ethylene, MAP kinase, Pathogen-triggered immunity

Poster #411. Understanding strigolactone signalling by the use of chemical genetics (Submission 492)
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Chemical genetics employs small molecules that reversibly alter protein function in order to study a biological process of interest. Here, we use a chemical genetics approach in Arabidopsis thaliana to get a better understanding in the molecular mechanism of strigolactone (SL) sensing and signaling. Strigolactones (SLs) are a novel group of phytohormones that were initially discovered because of their role in rhizosphere interactions (Xie & Yoneyama, 2010). Nowadays, SLs have become a cutting-edge topic in plant biology and agronomy, with great potential in modern agriculture, because of their various physiological responses. Nevertheless, much still needs to be discovered about the SL signaling cascades that are leading to their various physiological effects. A Chembridge DIVERsetTM library containing 12,000 structurally diverse chemical compounds was used for screening, in order to identify compounds that interfere with the SL mediated inhibition of hypocotyl elongation in red light (Nelson et al, 2011; Shen et al, 2007). Under normal circumstances, SLs cause a 50% reduction of hypocotyl length and we screened for compounds that prevent this effect. This resulted in the identification of 490 compounds. To reduce the number of compounds to be confirmed, they were clustered according to structural resemblance and one representative compound for each group was selected for further confirmation. From the selected 168 compounds, 58 could be confirmed, from which the compounds with a proaustin-like structure were discarded (Savaldi-Goldstein et al, 2008). This resulted in 30 compounds that were retained for further analysis. Here, a secondary screen will be discussed that was used to discard compounds that modulate hypocotyl length without affecting SL signaling. In addition, the effect of the retained compounds on various other SL dependent physiological responses, such as the effect on root system architecture and on germination will be shown.

Keywords: Strigolactones, chemical genetics, phytohormones
**Signal Transduction, Signal Integration**

**Talk #412. Phosphorylation of a bZIP transcription factor triggers metabolic reprogramming in acclimation to low energy stress** (Submission 15)
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Protein phosphorylation is a key regulatory principle enabling fast and reversible regulation of a vast number of cellular activities. In response to stress conditions cellular metabolism needs to be switched from normal growth to stress response, for example by the synthesis of protective or defense compounds. We found a bZIP transcription factor to be phosphorylated at multiple sites depending on the energy status of the cell. To identify the kinase(s) that phosphorylate this factor, we applied affinity purification combined with in-gel kinase assays and MS/MS to identify the relevant kinases. Co-localization and interaction of the identified kinases was confirmed by different means and direct phosphorylation assays were done to attribute the different sites to the identified kinases. Metabolic profiling and gene expression studies were done to elucidate functional consequences. Of the seven identified in vivo phosphorylation sites, three could be attributed to a SnRK1 kinase and two to a Ca2+-dependent kinase. Only one site targets the central bZIP domain and thereby could affect DNA-binding. The other sites affect hetero-dimerization with other bZIP factors and reporter gene assays in protoplasts revealed that the phosphorylation affects expression of key genes involved in amino acid metabolism. We propose that targeting this bZIP factor by different protein kinases at different sites occurs under different stress conditions and enables fast metabolic reprogramming by changing the specificity of heterodimerization with other bZIP factors.

Keywords: Protein phosphorylation, SnRK kinase, Primary Metabolism, Stress acclimation, Metabolic reprogramming

**Poster #413. Complex mechanisms of cell expansion in dark grown hypocotyl requires functional AUXIN BINDING PROTEIN 1** (Submission 36)
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Cell expansion is an increase in cell size and plays an essential role in plant growth and development. Phytohormones and the primary plant cell wall play major roles in the complex process of cell expansion. In shoot tissues, cell expansion requires functional AUXIN BINDING PROTEIN1 (ABP1) but the mechanism by which ABP1 affects expansion remains unknown. We performed multi-level analysis of the effect of functional inactivation of ABP1 on hypocotyl growth and revealed rapid and major changes in gene expression via a modulation of the SCFTIR1/AFB pathway as well as complex modifications of cell wall structure (mainly hemicellulose xyloglucan structure and cellulose microfibrils organization). Cortical microtubules and cellulose microfibrils are known to form a continuum, the organization of cellulose microfibrils resulting from oriented synthesis driven by oriented cortical microtubule. Both were found to be disturbed after inactivation of ABP1. By manipulating the capacity of hypocotyl cells to expand by acting on hemicellulose xyloglucan remodeling in seedlings inactivated for ABP1, we found as anticipated that stimulation of cell expansion imposes cellulose microfibril reorganization but more surprisingly induces also reorientation of cortical microtubules, suggesting reciprocal interdependency between both structures. Our data suggest that mechanical forces resulting from cell expansion affects cortical microtubule organization via the continuum with the cellulose.

Keywords: ABP1, cell expansion, signaling, transcriptional regulation, cell wall, mechanical forces

**Poster #414. Salicylic acid induces conformational remodeling in C-termini of NPR family proteins** (Submission 38)
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NON-EXPRESSOR OF PR GENES1 (NPR1; acronym NIM1) is the central regulator of salicylic acid (SA)-induced
defense responses and likely other aspects of physiology through its role as a Ca²⁺ effector. And have been implicated in Ca²⁺ signaling related to various physiological processes such as pathogen defense, development and thermotolerance. Furthermore, the anticipated mechanism is in accordance with the role of NPR1 as node in the SA signaling network (Arabidopsis Interactome Mapping Consortium, 2011) and with the suggested functions of NIMIN proteins (Hermann et al., 2013), which, together with TGA transcription factors (Zhang et al., 1999), are part of the NPR1 transcription complex (Weigel et al., 2005). Of note, SA perception and binding of SA-inducible NIMIN1 and NIMIN2 proteins engage nearby domains in NPR1. As a consequence, the NPR1 C-terminus can adopt different shapes, thus spurring differential contacts with transcription factors resulting in activation of NPR1-dependent genes according to the ambient SA concentration (Hermann et al., 2013).

Keywords: NIMIN, NPR1, NPR3, NPR4, salicylic acid receptor, TGA transcription factor

Poster/Talk #415. Molecular basis for AUXIN RESPONSE FACTOR protein interaction and the control of auxin response repression (Submission 49)

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In plants, the AUXIN RESPONSE FACTOR (ARF) transcription factor family regulates gene expression in response to auxin. In the absence of auxin, ARF transcription factors are repressed by interaction with AUXIN/INDOLE 3-ACETIC ACID (Aux/IAA) proteins. Although the C termini of ARF and Aux/IAA proteins facilitate their homo- and heterooligomerization, the molecular basis for this interaction remained undefined. The crystal structure of the C-terminal interaction domain of Arabidopsis ARF7 reveals a Phox and Bem1p (PB1) domain that provides both positive and negative electrostatic interfaces for directional protein interaction. Mutation of interface residues in the ARF7 PB1 domain yields monomeric protein and abolishes interaction with both itself and IAA17. Expression of a stabilized Aux/IAA protein (i.e., IAA16) bearing PB1 mutations in Arabidopsis suggests a multimerization requirement for ARF protein repression, leading to a refined auxin-signaling model.

Keywords: auxin signal transduction, X-ray crystallography, protein-protein interactions

Poster #416. rdd1, the suppressor of Arabidopsis cyclic nucleotide-gated ion channel mutant dnd1, revealed that AtCNGC2 and AtCNGC4 work in the same signaling pathway to regulate pathogen defense and salicylic acid-independent floral transition. (Submission 72)

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Arabidopsis thaliana cyclic nucleotide-gated ion channels (CNGCs) form a large family consisting of 20 members and have been implicated in Ca²⁺ signaling related to various physiological processes such as pathogen defense, development and thermotolerance. The null mutant of AtCNGC2, "defense, no death" (dnd1) exhibits autoimmune phenotypes, while it is impaired in mounting the hypersensitive response (HR), which is a hallmark of effector-triggered (a.k.a. R-gene mediated) resistance. It has been suggested that AtCNGC2 is involved in defense responses and likely other aspects of physiology through its role as a Ca²⁺-conducting channel.
However, the downstream signaling components and its relation with AtCNGC4, which is the closest paralog of AtCNGC2, remain elusive. Despite the fact that cngc4 mutants display almost identical phenotypes to those seen in cngc2 mutants, not much is known about their relationship. Here, we report the isolation and characterization of the Arabidopsis mutant, repressor of defense no death 1 (rdd1), obtained from a suppressor screen of a T-DNA insertion knockout mutant of AtCNGC2 in order to identify downstream components of dnd1-mediated signal transduction. rdd1 suppressed the majority of dnd1-mediated phenotypes and also suppressed the dnd1-mediated late flowering phenotype that was discovered in this study. Our genetic analysis conducted to elucidate the relationship between AtCNGC2 and AtCNGC4 indicates that RDD1 is also involved in AtCNGC4-mediated signal transduction. Bimolecular fluorescence complementation (BiFC) analysis suggests that AtCNGC2 and AtCNGC4 are likely part of the same channel complex. In addition, the late flowering phenotype of dnd1 was found to be salicylic acid independent. It has been known that accumulation of salicylic acid promotes early flowering, but dnd1 shows delayed flowering despite its high levels of salicylic acid. Our double mutant analysis with a salicylic acid biosynthesis and signaling mutants indicate that this late flowering phenotype of dnd1 is SA independent. On the other hand, another CNGC-related autoimmune mutant, cpr22, that accumulates high salicylic acid levels displayed early flowering, as expected. This data indicates a unique role of AtCNGC2 and likely AtCNGC4 in flowering that is not related to salicylic acid signaling.

Keywords: cyclic nucleotide-gated ion channels, CNGCs, salicylic acid, AtCNGC2, AtCNGC4, dnd1, floral transition

Poster #417. BES1 serves as a substrate of MAX2 and a negative regulator of the strigolactone signaling pathway to regulate shoot branching in Arabidopsis (Submission 77)
Yuan Wang¹, Shiyong Sun², Wenjiao Zhu¹, Xuelu Wang²
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Strigolactones (SLs) are new terpenoid plant hormones, which were originally discovered as stimulants for germination of parasite weeds. Recent studies demonstrate that SLs coordinate with auxin and cytokinins to modulate the development of plant lateral branches and shape the plant. Two critical components have been identified to be involved in SL signal pathway, including receptor AtD14/DAD2/D14 and an F-box protein MAX2/RMS4/D3. However, the substrates of MAX2 and the biochemical and molecular mechanism of the SL signal transduction have been major mysteries in the field. In this study, we found a gain of function mutant of an essential transcriptional effector in brassinosteroid (BR) signal pathway, bes1-D, has more rosette branches and longer hypocotyl, which are similar to a loss of function mutant of MAX2. In addition, the bes1-D mutant also exhibits similar SL insensitivity in inhibition of shoot branching and hypocotyl elongation with max2-1. Through screening the MAX2 interacting protein, we found MAX2 is able to interact with BES1 family protein, and both phosphorylated and dephosphorylated BES1 can be associated with MAX2. The further ubiquitination assay and cell free degradation assay showed that MAX2 is required for the ubiquitination and degradation of BES1. Moreover, the SL receptor also mediates the BES1 degradation. However, unlike the GA signal pathway, SL doesn't enhance the interaction between MAX2 and BES1, but promotes the MAX2-dependent degradation of BES1 protein. We also construct the BES1-RNAi/max2-1 double mutant and found that depletion of BES1 and its homologues would suppress shoot branching of max2-1. These evidences suggest that BES1 protein acts as a negative component downstream of MAX2 to deliver the SL signal. This study established an SL signaling cascade from the putative receptor to downstream transcription factors, paving the way for deeply understanding mechanism of how the SLs regulate the plant growth and development. In addition, we demonstrated that the SLs and BRs signaling pathways distinctly regulate the same transcription factor BES1 to control specific developmental processes.
(Wang et al., 2013, Developmental Cell 27, 681-688)

Keywords: Strigolactones, Brassinosteroids, BES1, MAX2, Branching, Protein degradation, Signal transduction

Poster #418. Nitric oxide binds to and modulates the activity of a pollen specific Arabidopsis diacylglycerol kinase (Submission 104)
Aloysius Wong¹, Lara Donaldson¹, Maria Portes², Jose Feijo², Chris Gehring¹

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Nitric oxide (NO) is an important signaling molecule in plants. In the pollen of Arabidopsis thaliana, NO causes re-orientation of the growing tube and this response is mediated by 3',5'-cyclic monophosphate (cGMP). However, in plants, NO-sensors have remained somewhat elusive. Here, we present work on an NO-binding candidate, Arabidopsis thaliana DIACYLGLYCEROL KINASE 4 (ATDGK4; AT5G57690). In addition to the annotated diacylglycerol kinase domain, this molecule also harbors a predicted heme-NO/oxygen (H-NOX) binding site and a guanylyl cyclase (GC) catalytic domain which we have identified based on the alignment of functionally conserved amino acid residues across species. We have constructed a 3D model of the molecule, from which we have estimated the locations of the kinase catalytic center, the ATP-binding site, the GC and H-NOX domains. We have also modeled docking of ATP to the kinase catalytic center. The recombinant ATDGK4 demonstrated kinase activity in vitro, catalyzing the ATP-dependent conversion of sn-1,2-diacylglycerol (DAG) to phosphatidic acid (PA). This activity is inhibited by the mammalian DAG kinase inhibitor R59949 and importantly also by the NO donors diethylamine NONOate (DEA NONOate) and sodium nitroprusside (SNP). Recombinant ATDGK4 also has GC activity in vitro, catalyzing the conversion of guanosine-5'-triphosphate (GTP) to cGMP. The catalytic domains of ATDGK4 kinase and GC may be independently regulated since the kinase but not the GC, is inhibited by NO while Ca2+ only stimulates the GC. It is likely that the DAG kinase product, PA, causes the release of Ca2+ from the intracellular stores and Ca2+ in turn activates the GC domain of ATDGK4 through a feedback mechanism. Analysis of publicly available microarray data has revealed that ATDGK4 is highly expressed in the pollen. Here, the pollen tubes of overexpressing atdgk4 recorded slower growth rates than the wild-type (Col-0) and importantly, they showed altered NO responses. Specifically, the overexpressing atdgk4 pollen tubes have growth rates that were less affected by NO and showed reduced bending angles when challenged by an NO source. Further works on atdgk4 knockout/knockdown mutants will reveal the biological functions of ATDGK4 in NO and/or cGMP signaling in the pollen, and in the broader fertilization process.

Keywords: Nitric oxide, cGMP, Pollen, H-NOX, Guanylyl cyclase (GC), Diacylglycerol kinase

**Poster #419. A plasma membrane-localized kinase buffers plant immunity by modulating BIK1 protein levels** (Submission 113)
Jacqueline Monaghan¹, Susanne Matschi², Oluwaseyi Shorinola¹, Hanna Rovenich¹, Alexandra Matei¹, Cecile Segonzac¹, Frederikke Gro Malinovsky¹, John Rathjen², Dan MacLean¹, Tina Romeis², Cyril Zipfel¹
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Perception of pathogen-associated molecular patterns (PAMPs) triggers a phosphorylation relay leading to PAMP-triggered immunity (PTI). Despite an increasing knowledge of PTI signaling, how immune homeostasis is maintained remains largely unknown. Here we report a novel forward-genetics screen to identify modifier of bak1-5 (mob) mutants with regained immune responses in the immune-deficient bak1-5 background. We identified and characterized MOB1, a plasma membrane-localized kinase, as a negative regulator of immunity. Detailed genetic analyses demonstrate that MOB1 attenuates immune responses triggered by several PAMPs and negatively regulates anti-bacterial immunity. MOB1 interacts with and phosphorylates the plasma membrane-associated cytoplasmic kinase BIK1, which is an important convergent substrate of multiple pattern recognition receptor (PRR) complexes. We find that BIK1 protein accumulation is lower in MOB1 over-expression lines and higher in mob1 mutants, and BIK1 levels can be restored upon inhibition of the 26S proteasome. Our results suggest a constitutive regulatory mechanism that buffers immune signaling by controlling the turnover of a key kinase activated downstream of numerous PRR complexes.

Keywords: Plant immunity, Phosphorylation, Protein turnover

**Poster/Talk #420. A receptor-like protein links cell wall surveillance with brassinosteroid signaling** (Submission 114)
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Plant growth depends to a large extent on the physico-chemical properties of the cell walls, which dynamically adapt to internal and external cues. This adaptation involves feedback signaling, linking information on the integrity of the wall with intracellular growth-regulating processes. However, very little is known about the nature of these pathways and how signals are transduced to the cytosol. The brassinosteroid (BR) hormone signaling pathway is a central regulator of plant morphogenesis, as indicated by the large amount of BR-responsive cell wall-related genes and the severe growth defects of BR mutants. Recently, we have shown that interference with the major cell wall polysaccharide pectin triggers activation of BR signaling, which in turn orchestrates a compensatory response involving cell wall remodeling. In the absence of BR-mediated feedback signaling, altered pectin modification severely compromises cellular integrity, ultimately resulting in cell rupture. Through a forward genetic screen, a receptor-like protein (CNU2) was identified which conveys information on cell wall state to BR signaling through direct interaction with the BR receptor complex. Using genetic, biochemical, and transcriptomic approaches, we demonstrate that CNU2 is not itself a component of the BR pathway, but instead conditionally activates hormone signaling when triggered by cell wall-related cues. Furthermore, activation of the BR pathway involves CNU2 phosphorylation, which is modulated upon cell wall modification. Taken together, feedback information from the cell wall is able to modulate BR signaling, presumably to ensure cell wall homeostasis during cell elongation.

Keywords: cell wall, signal transduction, brassinosteroids, growth control

**Poster #421. A cell wall-associated, Pro-rich receptor-like kinase RHS10 negatively modulates root hair growth** (Submission 146)
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Root hairs are tip-growing tubes that emerge from the root trichoblast cell. Arabidopsis root hairs have been a model system for studying the mechanisms of cell morphogenesis. In plants there are hundreds of receptor-like protein kinases (RLKs) which are involved in diverse development processes. So far, few RLKs have been implicated in root hair morphogenesis. The RHS10 (AT1G70460) RLK has a Pro-rich N-terminal region and is described as a member of the PERK (Pro-rich extensin-like receptor kinase) family. As PERKs have extensin-like motifs, the extracellular domain of RLKs might mediate cell wall-related signals. We characterized the RHS10 function in the root hair system by analyzing the extensin domain, functional orthologous relationship, ROS (reactive oxygen species)-mediated signaling, and the root hair growth rate. The RHS10 loss-of-function mutant grew longer root hairs than wild type, whereas its overexpression greatly inhibited root hair growth. The serial deletion analysis of the RHS10 extensin domain, using the root hair assay system, showed that a minimum number of Pro residues are required for RHS10 to inhibit root hair growth. Root hair-specific overexpression of rice and poplar RHS10 orthologs in Arabidopsis considerably inhibited root hair growth as seen with AtRHS10 overexpression, indicating that the RHS10 function in root hair development has been conserved in angiosperms. ROS has been known to be necessary for root hair tip growth. Consistently with its hair-inhibitory function, RHS10 overexpression reduced ROS accumulation in the root. The yeast two-hybrid screening revealed a ribonuclease as a downstream interactor of RHS10, which also acts negatively on root hair growth. Our results suggest that RHS10 mediates cell wall-related signals to maintain proper root hair length at least by regulating RNA catabolism and ROS accumulation.

Keywords: root hair, cell wall, Pro-rich receptor kinase, extra-cellular signal

**Poster #422. Stimulus perception and signal transduction during plant cell wall integrity maintenance** (Submission 148)
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The plant cell wall represents a part of the first line of defense against biotic and abiotic stress while at the same forming a crucial component of plant development. In order to fulfill its diverse biological functions it needs to maintain its functional integrity during interaction with the environment and cell morphogenesis. This objective is achieved through tightly regulated changes in cell wall composition and structure as well as adaptive
modifications of cellular metabolism upon detection of cell wall integrity (CWI) impairment. The available evidence suggests that at least three, qualitatively different sensory and signaling mechanisms are involved in CWI maintenance. Here we will present novel insights into the early signaling events upon impairment of CWI and discuss how Receptor Like Kinases (THESEUS, HERKULES1/2, FERONIA, PEPPER1/2, FEI1/2, WAK2) and different signaling molecules like NO, JA and Ca2+ modulate the responses to cell wall integrity (CWI) impairment.

Keywords: Cell wall integrity, Receptor Like Kinase, Jasmonic acid signaling, Turgor Pressure Perception

Poster #423. The IDA peptide regulating floral abscission triggers an oxidative burst by direct binding to the HSL2 receptor (Submission 149)
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Small signaling peptides play an important role in plant growth and development. INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) encodes a small secreted peptide necessary for the regulation of floral organ abscission which is dependent on the two leucine-rich repeat receptor-like kinases (RLKs) HAESA (HAE) and HAESA-LIKE 2 (HSL2) to exert its function (Stenvik et al. 2008, Cho et al.2008).
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 of the peptide. Here we show that a dodecapeptide IDA peptide modified with a hydroxyproline induces a rapid release of reactive oxygen species (ROS) in an oxidative burst assay in N. benthamiana upon binding and activation of the HSL2 receptor. These results, showing direct biochemical interaction between IDA and HSL2, substantiate that these proteins form a peptide-receptor system.
Abscission has been shown to be accompanied by production of reactive oxygen species (ROS) in other species. Thus, a link between the IDA-HSL2 induced ROS production in N. benthamiana and ROS in floral abscission in Arabidopsis will be presented.

Keywords: IDA, HAE, HSL2, RLK, signaling, oxburst, abscission

Talk #424. Regulation of low oxygen signaling in plants (Submission 162)
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Oxygen levels within plant tissues vary dramatically as a function of oxygen availability in the environment and cell metabolism. Restricted oxygen supply is often associated to heavy rains or flooding events and is a major cause for losses in crop-yield worldwide. Since plants require oxygen for ATP production via oxidative phosphorylation, they also need to rapidly adapt to insufficient oxygen availability by activating escape or tolerance strategies. The group VII ERF transcription factors play a major role in the activation of these processes. We described how, in Arabidopsis thaliana, the ERF-VII activity is restricted in an oxygen-dependent manner by proteasomal degradation and membrane sequestration under aerobic conditions while, under low oxygen conditions, this blockage is released. We recently discovered that two Plant Cysteine Oxidase enzymes (PCO1 and PCO2) redundantly control ERF-VII activity by using molecular oxygen to generate the signaling moiety that trigger proteolysis following the N-end rule pathway. Since the two PCO genes are themselves targets of ERF-VII, they also contribute to rapid switch-off of the anaerobic response after reoxygenation in feed-back loop fashion. The PCO-ERF-VII circuitry constitutes a valuable resource for the improvement of crop tolerance to flooding and low oxygen conditions.

The Floral Abscission Zone as an Organ Boundary (Submission 176)
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Small peptides play an important role in plant growth and development. INFLORESCENCE DEFICIENT IN ABSCISSION (IDA) encodes a peptide ligand necessary for the regulation of floral organ abscission. 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 (Stenvik et al. 2008). A dodeca-hydroxyprolinated IDA peptide derived from the conserved domain signals through the receptor-like kinases (RLK) HAESA (HAE) and HAESA-LIKE 2 (HSL2) by binding and activating HSL2 (Butenko et al. Plant Cell in press). Genetic interaction studies show that IDA signaling activates a MITOGEN-ACTIVATED PROTEIN KINASE cascade and regulates the action of the homeobox transcription factors, KNOTTED-LIKE FROM ARABIDOPSIS THALIANA1 (KNAT1), KNAT2 and KNAT6 to induce separation between abscission zone (AZ) cells (Cho et al. 2008; Shi et al. 2011). Here we will present results showing that downstream signaling components of the IDA pathway are common to those specifying boundary domains during plant development. We provide genetic evidence that the BLADE ON PETIOLE (BOP) genes BOP1 and 2 previously reported to be involved in abscission zone (AZ) formation (Hepworth et al. 2005, Nordberg et al. 2005) in addition are acting downstream of IDA and regulate KNAT2 and KNAT6. Microarray data suggests the involvement of LBD proteins in the control of floral abscission. We are currently investigating how selected LBD genes are regulated by IDA signaling and BOP1 and 2.

Identification of SUMO Footprints Using Plants Blocked in PolySUMO Chain Formation (Submission 184)
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The post-translational modification of proteins by Small Ubiquitin-like Modifier (SUMO) is an essential cellular process in eukaryotes, with genetic studies indicating that it plays a critical role in protection against numerous environmental challenges. Using proteomic methods, we recently identified hundreds of SUMO targets in Arabidopsis that are involved in a wide array of nuclear events, including transcription, chromatin remodeling, and RNA biology. Upon exposure to heat shock and other abiotic stresses, the SUMOylation level of some of these proteins increases dramatically and reversibly, especially for those involved in RNA-dependent processes. In addition, modification of SUMO by itself, as well as by ubiquitin, was detected. These modifications are dynamic and vary under different stresses, suggesting that SUMOylation serves as a rapid and robust signaling mechanism that integrates multiple processes into a coherent defense response. To map the sites of SUMO conjugation, we are using a novel technique to detect SUMOylation footprints by mass spectrometry (MS) that employs a lysine-null (K0) SUMO1 construct combined with an alternative peptidase cleavage prior to trypsinization. This method allows for the exclusive purification of peptides containing a SUMOylation footprint, which enhances MS mapping of conjugation sites by reducing sample complexity. By comparing SUMOylation sites between normal and stressed conditions, the potential dynamics of this modification can be studied. Importantly, we found that the lethal SUMO1/2 double mutant can be rescued with K0 SUMO1, indicating that this lysine-less variant is functionally normal. The wild-type activity of K0 SUMO1, which presumably cannot be modified with either SUMO or ubiquitin, implies that polySUMO chain formation and SUMO ubiquitylation are not essential. Ultimately, mapping of SUMOylation sites will accelerate in-depth studies of individual SUMO targets and their role in the stress response.

Keywords: N-end Rule Pathway, Ethylene Responsive Factors, Low oxygen signalling, Cysteine oxidation, Proteasome
Poster #427. Redox control of plant translation (Submission 195)
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In flowering plants, eIF4E and plant-specific homolog eIFiso4E participate in translation initiation by recognizing the 7-methylguanosine (m7G) cap on the 5' end of mRNA and cooperating with many other translation initiation factors to recruit and assemble the ribosome for translation of the mRNA into protein. The simultaneous deletion of the two cap-binding proteins in Arabidopsis thaliana is lethal, and each individually contributes to plant viability in combination with other subunits (eIF4G or eIFiso4G) to form the eIF4F and eIFiso4F complex. The crystal structure of wheat eIF4E shows that two plant-specific cysteines (C151, C133) are capable of forming a disulfide bond. This disulfide bond appears to may influence the affinity of eIF4E for the m7G cap. Redox-thiol labeling reveals a disulfide bond in eIFiso4E, suggesting that eIFiso4E (and likely eIF4E) may respond to the redox state of the cell. We observe that a cysteine-to-serine eIFiso4E mutant protein (C\textsuperscript{\textgreater}S), lacking the ability to form the disulfide bond, can rescue the lethal phenotype of eIFiso4E x eIF4E double knockout Arabidopsis plants in normal growth conditions and the C\textsuperscript{\textgreater}S mutant appears more sensitive to oxidative stresses than wild-type plants or eIFiso4E x eIF4E double knockout Arabidopsis plants rescued with wild-type eIFiso4E. In addition, the signaling of oxygen reactive species propagation is altered in wounded C\textsuperscript{\textgreater}S mutants. This observation suggests that the disulfide bond of eIFiso4E (and likely eIF4E) contributes to plant viability and may have a role in regulation of translation during plant development and in response to environmental conditions that alter the cellular redox status.

Keywords: eIFiso4E, translation, redox

Poster/Talk #428. Inhibition of Developmental and Stress-Induced Leaf Senescence by Cytokinin Response Factor 6 (Submission 200)
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Cytokinin Response Factor 6 (CRF6) is a member of the CRF sub-group of AP2/ERF transcription factors. CRFs comprise a single monophyletic group of proteins found in all land plants many of which, including CRF6, are transcriptionally induced by cytokinin. We have recently shown that CRF6 acts as a negative regulator of leaf senescence in Arabidopsis, mediating the well-known senescence delaying effect of cytokinin. Interestingly, CRF6 is also rapidly induced by numerous stress conditions and has been shown to be a target of ANAC transcription factors as part of a mitochondrial retrograde signal of oxidative stress. We have hypothesized that CRF6 may act as central regulator of stress-induced senescence responses, integrating whole plant/organ level signals (cytokinin) and intercellular signals (mitochondrial retrograde regulation). In agreement with this role, we demonstrate here that overexpression of CRF6 leads to enhanced tolerance to a variety of senescence inducing stress conditions. Supporting the functional conservation of CRF6; transient expression of the tomato (Solanum lycopersicum) ortholog, SICRF5, delays senescence in leaves of Nicotiana benthamiana. In order to determine the process(es) downstream of CRF6 responsible for increased stress-tolerance, as well as delayed leaf senescence we have performed transcriptome analyses of mutant and over-expressing transgenic lines under standard and senescence inducing (oxidative stress) conditions. The results of these experiments, identification of downstream transcriptional targets of CRF6 and preliminary characterization of these targets will be presented and discussed in the context of cytokinin signaling, leaf senescence, and stress response.

Keywords: cytokinin, abiotic stress, senescence, mitochondrial retrograde regulation, cytokinin response factor, transcription factor

Poster #429. Metabolism and inter-tissue growth coordination: lessons from low-cell-density mutants (Submission 207)
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Plants are sessile organisms that cannot escape adverse environmental conditions, but can continuously adapt to their environment by altering local patterns of growth and development. One remarkable aspect of such plasticity
is the ability of plants to maintain coordination in cell division and expansion in an organ across multiple cell layers despite the dramatic changes in the overall organ size and shape dictated by the environment. To study inter-tissue growth coordination, we manipulated cell division in Arabidopsis leaves in a tissue-specific manner using transgenic approaches. Our results indicate that a defect in cell division in mesophyll can be relayed to the epidermis and vice versa, in both cases leading to small leaves. These findings confirm existence of an active growth coupling mechanism between tissues, but its molecular nature remains largely unresolved. We are taking advantage of a unique class of uncoupled mutants in Arabidopsis that are disrupted in the communication between the epidermis and mesophyll in leaves. In these mutants, the epidermal cell layer develops normally, but the cell division in the metabolically compromised mesophyll is impaired. In the absence of a functional growth synchronization mechanism, the epidermis overgrows with respect to the inner tissues, leading to reduced inner cell density and large air spaces between the mesophyll cells. Cloning of a genetic suppressor of one uncoupled mutant suggests that biomechanical forces play a critical role in the coordination of growth between the epidermis and mesophyll in leaves.

Keywords: growth coordination, cell-cell communication, low cell density

Poster #430. The receptor-like kinase FERONIA mediates Ca2+ signaling and growth responses in mechanically stimulated Arabidopsis roots (Submission 211)
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Among the myriad cues that constantly inform plant growth and development, mechanical forces are unique in that they are an intrinsic result of cellular turgor pressure and also imposed by the environment. While the key role of mechanical forces in shaping plant architecture from the cellular level to the level of organ formation is well established, the components of the early mechanical signal transduction machinery remain to be defined at the molecular level. The earliest cellular responses to mechanical stimulation typically involve a rapid elevation of cytosolic Ca2+ levels and Ca2+-dependent signal transduction appears central to many plant mechanoresponses. Here we show that an Arabidopsis mutant lacking the receptor-like kinase FERONIA shows severely altered Ca2+ signaling and growth responses to different forms of mechanical perturbation. Ca2+ signals are either abolished or exhibit qualitatively different signatures in fer mutants exposed to local touch or bending stimulation. Furthermore, mechanically-induced upregulation of known TCH genes is significantly decreased in fer mutants. In addition to these defects in mechanical signaling, fer mutants also exhibit growth phenotypes consistent with impaired mechanical development, including biased root skewing, an inability to penetrate hard agar layers and abnormal growth responses to impenetrable obstacles. Based on these results, we propose that FER is a key regulator of mechanical Ca2+ signaling and that FER-dependent mechanical signaling functions to regulate growth in response to mechanical forces.

Keywords: Mechanical signaling, Receptor-like kinase, Ca2+ signaling, Root growth

Poster #431. Identification of OST1 Target Proteins by Mass Spectrometry-based Kinase Client Assay Method (Submission 216)
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Abscisic acid (ABA) plays regulatory roles in many physiological and developmental processes in plants. ABA promotes stomatal closure and inhibits stomatal opening to minimize water loss during drought stress. Arabidopsis OST1 (OPEN STOMATA1) is a Ser/Thr protein kinase and a key positive regulator in guard cell ABA signaling. Recently we have shown that OST1 is a limiting component in guard cell ABA signaling (Acharya et al, doi: 10.111/nph.12469).

Only a few OST1 target proteins are known. In addition, the relationship of OST1 with other proteins that are vital components in guard cell ABA signaling is largely unknown. We used the kinase client assay (KiC assay) method, a label-free mass-spectrometry-based approach, to identify new client proteins of OST1 and to investigate the relationship between OST1 and other ABA signaling proteins. This method employs three steps for identification
of putative clients of a specific purified protein kinase: (1) in vitro peptide kinase assay of synthetic peptide cocktails (11-20 mers) (peptide sequences that are known or hypothesized to be phosphorylated in vivo during specific developmental or stress conditions); (2) liquid chromatography (LC)-tandem MS (MS/MS) analysis; (3) data analysis and interpretation.

Screening of a library containing ~2200 synthetic peptides using recombinant OST1 identified 64 putative OST1 client proteins that belong to different functional groups including proteins that are annotated to function in signal transduction, metabolic process, nucleic acid binding, development, and other biological processes. Among these, 42% of the putative OST1 clients are plasma-membrane localized. This study also identified OST1-mediated transphosphorylation of 37 peptides which correspond to 18 different proteins known to play key roles in guard cell ABA signaling. To date, we have validated the KiC data by BiFC assays for ~30% of the OST1 clients that we have identified.

The findings of this study not only identified novel target proteins for OST1 but also revealed that OST1 can phosphorylate many key protein-components of ABA signaling in guard cells.

Keywords: Abscisic acid (ABA), OST1 (OPEN STOMATA1), Protein kinase, KiC assay(kinase client assay), Client proteins

Poster #432. Nitric oxide production in Arabidopsis thaliana (Submission 252)
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Over the last decades, nitric oxide (NO) has emerged as a crucial signaling molecule in plants, being involved in developmental processes as well as in the response to abiotic and biotic stresses. Despite its important roles in plant biology, the origin of NO in plants remains unclear. To date, two main routes for NO production have been described in higher plants. Some evidences demonstrated the existence of an oxidative pathway similar to the production of NO by nitric oxide synthase (NOS) observed in animals, even though no homologous enzymes have been found in higher plants. Secondly, nitrite can be reduced to NO, notably the well characterized nitrate reductase enzyme.

Our work aims to identify new proteins involved in the NO production in Arabidopsis via a candidate-approach. We assayed the NO production in about 25 Arabidopsis thaliana T-DNA insertion lines impaired in the expression of selected candidate-genes, based on the recent literature. Among the candidates proteins were aldehyde oxidases, amine oxidases and polyamine oxidases. In these lines NO production was measured in seedlings roots, stained with an NO-specific fluorescent dye (DAF-FM DA), after treatment with an elicitor. From this screen, several mutants presented a significant reduction of NO production after treatment, as compared to the wild type. A selected candidate, belonging to the group of amine oxidases, displayed a 30% decrease in fluorescence and is currently under characterization both in vivo and in vitro. The corresponding mutant line displays also alterations in two physiological processes in which NO plays a crucial role: ROS production after elicitor treatment and a reduced resistance against Pseudomonas syringae.

Keywords: nitric oxide, amine oxides, reactive oxygen species

Poster #433. Metabolites with anti-abscisic acid activity (Submission 272)
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Stomatal pores regulate the balance of water use efficiency and CO2 entry for photosynthesis. Declining soil-water potential in plants induces the synthesis of abscisic acid (ABA), which then triggers stomatal closure to conserve tissue moisture. Closed stomates, however, also create a dilemma disrupting the large CO2 influx required for net photosynthesis. The depleting CO2 in the plant will in turn bias stomatal opening by suppressing ABA sensitivity, which then aggravates transpiration further. We have investigated the molecular basis of how C3 plants resolve this H2O-CO2 conflicting priorities created by stomatal closure. Here, we have identified in
Arabidopsis thaliana an early drought-induced acetyltransferase homolog, which can slow or reverse ABA-mediated stomatal closure. Evidence from genetic, biochemical and physiological analyses revealed that this protein does so by acetylating the metabolite, 1,3-diaminopropane (DAP), thereby turning on the latter's intrinsic activity. Acetylated DAP triggers plasma membrane electrical and ion transport properties opposite to those by ABA. Thus in adapting to declining soil-water availability, acetyl-DAP could refrain complete stomatal closure to sustain CO2 diffusion to photosynthetic tissues and to ease stress recovery when water returns.

Keywords: metabolite switch, guard cell, abscisic acid, acetylation

**Talk #434. The role of Integrin-Linked Kinase 1 in perception and control of ionic signals during biotic and abiotic stress** (Submission 300)

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Bacterial pathogens of both plants and humans induce cellular responses within minutes of perception. These early responses are critical for activation of innate immunity and include kinase-mediated signal transduction and induction of ion transport. In plants, the identity of the cellular components that coordinate early signaling events with ion fluxes across the plasma membrane are currently unknown. Our work suggests that the Integrin-linked kinase 1 (ILK1) is a critical player in connecting these cellular events in Arabidopsis. Plants with defective ILK1 expression or kinase activity are more susceptible to bacterial pathogens and more tolerant to salt stress. ILK's role in stress responses is related to signaling since mutant lines have altered MAP kinase responses following bacterial or ionic stress treatments. ILK1 is also critical for the maintenance of cellular K⁺ ion fluxes immediately following stress induction and conversely, ILK1 is required for the perception Mn2+ specific signals during immunity. In combination with our data showing that ILK1 has an unusual preference for manganese (Mn2+) as a cofactor, this suggests that ILK1 functions as a sensor for Mn2+ fluxes. Together, our data demonstrate conservation of early signaling events between abiotic and biotic stress and support a role for metal ion fluxes as ILK1-dependent signals upstream of MAPK signal transduction.

Keywords: Abiotic stress, biotic stress, ionomics

**Poster #435. The role of CIPK8 in regulating nitrate response** (Submission 317)

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Our previous study showed that nitrate transporter CHL1 also function as a nitrate sensor regulating nitrate-induced transcriptional response. Like nitrate uptake, nitrate-induced transcriptional response also display two phases, a high-affinity phase and a low-affinity phase. Two phases are differentially regulated, as CIPK23 (a CBL-interacting protein kinase) is a negative regulator of the high affinity phase while CIPK8 is a positive regulator of the low-affinity phase, indicating that via the action of CIPK23 and CIPK8, CHL1 can detect nitrate concentration changes in the soil, and elicited different levels of transcriptional responses. In response to low nitrate (high-affinity level of nitrate), CIPK23 can phosphorylates the threonine 101 residue in the N-terminal half of CHL1. By this phosphorylation, the uptake activity of CHL1 can be switched between high- and low-affinity modes, and the gene expression level of the transcriptional response can be regulated. In this study, we investigated the role of CIPK8 in regulating the nitrate uptake and the nitrate signaling. Using yeast two-hybrid assay, we found that CHL1 have two CIPK8 binding sites in C-terminal half and the CHL1 central loop will block the interaction between CHL1 and CIPK8. Both CBL1 and CBL9 can increase the interaction between full length CIPK8 and central-loop deleted CHL1. These interaction data suggested that conformation changes in both CHL1 and CIPK8 are necessary for their interaction. Xenopus oocyte uptake activity assay showed that CIPK8, when co-expressed with CBL1 or CBL9, has opposite effects on the nitrate uptake activity of CHL1. Although cipk8-1 mutant was only defective in low affinity phase of primary nitrate response, the western analysis indicated that in response to low nitrate, CHL1 T101 can't be phosphorylated in cipk8-1 mutant. Our data suggested that CHL1 may have two CIPK8 binding sites; and through the cooperation between different CBLs, two sites has different effects in regulating the nitrate uptake activity and nitrate sensing ability of CHL1.
Keywords: nitrate, CHL1, CIPK

Poster #436. The SA-inducible CC-type glutaredoxin ROXY19/GRX480 might recruit the general transcriptional corepressors TPL/TPR to suppress JA/ET-responsive gene expression (Submission 329)
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The defense hormones salicylic acid (SA) and jasmonic acid (JA) and/or ethylene (ET) control plant responses against biotrophic and necrotrophic pathogens, respectively. In Arabidopsis thaliana, the three redundant class II TGA transcription factors TGA2, TGA5 and TGA6 play a role in both defense pathways. Despite their long-known involvement in SA-mediated defense responses, they are also essential for the activation of JA/ET-dependent mechanisms. The two hormone pathways are mutually antagonistic, allowing plants to coordinate their defense responses to particular biotic stresses. Consistently, exogenous application of SA increases the susceptibility of plants to necrotrophic attackers by suppressing the JA/ET defense response. This disease-promoting SA effect is abolished in tga256 mutant plants, pointing out that class II TGA factors are also involved in the SA-JA/ET hormone cross-talk. An interaction partner of the TGA class II factors, the SA-inducible CC-type glutaredoxin ROXY19/GRX480, might mediate the repressive effect on JA/ET-inducible genes. When ectopically expressed, ROXY19/GRX480 negatively regulates JA/ET-induced defense genes through an unknown mechanism that requires class II TGA transcription factors.
In a yeast-two-hybrid screen, we discovered the TIFY/ZIM domain protein TIFY8 to interact with ROXY19/GRX480. TIFY8 belongs to the same protein family as the JASMONATE-ZIM DOMAIN (JAZ) repressor proteins, which are involved in JA signaling. It differs from the JAZ proteins by lacking the Jas domain, which mediates interaction with the JA receptor CORONATINE-INSENSITIVE1 (COI1), an F-box protein necessary for the degradation of JAZ repressors in the presence of JA. Since TIFY8 can interact with NOVEL INTERACTOR OF JAZ (NINJA), which binds as an adaptor protein to the general transcriptional repressors TOPLESS (TPL) or TPL-related (TPR), we are speculating that TIFY8 bridges a repressor complex to ROXY19/GRX480. Yeast studies revealed that NINJA as well as ROXY19/GRX480 recruitment is mediated by the TIFY/ZIM domain of TIFY8. Using protein bridging assays in yeast, we were able to detect a ternary ROXY19/GRX480-TIFY8-NINJA complex. Currently, we are investigating if TIFY8-mediated co-repressor recruitment is involved in the suppressive effect of ROXY19/GRX480 on JA/ET-inducible genes and/or if it mediates the SA-JA/ET hormone cross talk in Arabidopsis plants.

Keywords: SA-JA/ET cross-talk, TGA transcription factors, CC-type glutaredoxins

Poster #437. Developmental Regulation of the GH3.17 Gene Modulating Auxin Levels in Arabidopsis (Submission 383)
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Auxins play pivotal roles in plant growth and development. Extensive studies have been carried out to elucidate a complex signal transduction network to direct plant growth and developmental process. However, the modulation of auxin levels for proper plant development is not well understood. We isolated a mutant with a severe dwarf phenotype from activation-tagged lines of Arabidopsis ecotype Columbia (Col-0). The mutant exhibited dwarfism, reduced lateral root number, and shortened hypocotyl length in the long light condition. The mutant was named short stem and leaves 1-Dominant (ssl1-D). TAIL-PCR and plasmid rescue experiments identified the location of T-DNA insertion in the intergenic region between At1g28120 and At1g28130. RT-PCR showed that At1g28130 was activated by the cauliflower mosaic virus (CaMV) 35S enhancer located in the T-DNA. At1g28130 corresponds to the GH3.17 gene that encodes an IAA-Asp conjugating enzyme with 609 amino acids belonging to the GH3 gene family. Recapitulation carried out by overexpressing At1g28130 (SSL1) under the control of CaMV 35S promoter confirmed that SSL1 was responsible for the dwarf phenotype of the mutant. These suggest that
the SSL1 proteins reduce concentrations of active IAA by conjugating IAA with aspartic acid (Asp), leading to the dwarf phenotype. The expression pattern analyzed by the SSL1::GUS transgenic line showed strong GUS activity in the root and the shoot meristems of seedlings, stamens of flowers, and the abscission zone of siliques. GFP-fused SSL1 was identified in the cytosol. The SSL1 gene was not induced by IAA, SA or ACC treatment. However, it was induced by ABA. Thus, these results suggest that the SSL1 enzymes modulate auxin levels in different tissues at the specific developmental stages.

Keywords: auxin, plant development, stress, hormones

**Poster #438. A MAP kinase pathway initiated by YDA regulates BR-mediated plant growth** (Submission 395)

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YODA (YDA) MAPKKK plays an essential role to control stomatal development in Arabidopsis. The yda knockout mutant showed strong stomatal cluster whereas the gain-of-function mutation of YDA (CA-YDA) completely abolishes stomatal development. The MAP kinase module involved in stomatal development includes YDA, MKK4/5, and MPK3/6. Previously, we showed that YDA is directly regulated by GSK3-like kinase BIN2 acting as a negative regulator in brassinosteroid (BR) signaling. In this study, we investigated the involvement of YDA-mediated MAP kinase pathway in BR signaling that regulates plant growth. Notably, the yda knockout mutant displays greatly reduced hypocotyl elongation. Failure of photosynthesis in homozygote CA-YDA due to complete loss of stomata caused the retardation of growth while heterozygote CA-YDA possessing normal stomata showed promoted hypocotyl elongation. When CA-YDA or YDA overexpression was introduced into bri1-5 or bin2-1 mutant, growth defects as well as BR-regulated gene expression in those mutants were restored. Interaction studies demonstrated that MKK4/5, but not YDA, interacts with and phosphorylates BZR1 in vitro, indicating that a signal from MAPKK in YDA pathway might be branched into BZR1-regulated gene expression. Our results suggest that an YDA-mediated MAP kinase pathway regulates not only stomatal development but also BR-regulated growth.

Keywords: MAPK, BZR1, YODA, Brassinosteroid signaling

**Poster #439. Arabidopsis BSU1 Family Members in Brassinosteroid signal transduction pathway** (Submission 396)

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A wide range of physiological processes of plants are essentially regulated by a steroidal plant hormone brassinosteroid (BR). Recently, a fully connected BR signal transduction pathway that shows signal relays from a cell surface receptor kinase to nuclear transcription factors has been established. Among BR signaling components, BR1 SUPPRESSOR 1 (BSU1) encodes a Kelch-repeat protein phosphatase, which functions as a positive regulator dephosphorylating GSK3-like kinase BIN2 in BR signaling. BSU1 is belonging to a small family of phosphatases that is composed of 4 homologues (BSUf; BSU1, BSL1, BSL2, and BSL3). Recent study demonstrated that BSUf inhibition of GSK3s is important to the regulation on not only cell growth but also stomatal development. In this study, we show that BSUf members form oligomer that is crucial for BR signaling. All four members of BSU1f were detected in mass spectrometry analysis for proteins immunoprecipitated by anti-YFP antibody from BSU1-YFP transgenic plants. Direct interaction between BSU1f members was confirmed by co-immunoprecipitation, yeast two-hybrid assays, in vitro pull down assay, and BiFC assay. Importantly, we found that a BSU1 mutant (BSU1d4) harboring deletion of short conserved region fails to form oligomers. Phenotypic analysis of transgenic bri1-5 overexpressing wild-type or BSU1d4 suggested that oligomerization of BSUf enhances BR signaling. Furthermore, it revealed that BSU1d4 activity dephosphorylating BIN2 is decreased compared with that of wild-type BSU1. These results demonstrate that oligomerization of BSUf members positively regulates the BR signal transduction pathway.
Keywords: BSU1, Oligomerization, Brassinosteroid

Poster #440. Role of NLP7 in primary nitrate response (Submission 398)
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Nitrate is a key nutrient for plant growth and development. It serves not only as a nitrogen source but also as a signal molecule to regulate gene expression, plant metabolism and development. Recent studies have identified several components involved in the nitrate signaling pathway; including NLP7 (Arabidopsis thaliana NIN-Like protein 7), a transcription factor. In Arabidopsis, there are nice NLP genes. Study of Marchive et al., showed that NLP7 is regulated by nitrate via a nuclear retention mechanism while study of Konishi and Yangisawa showed that RWP-RK domain of NLPs could bind to nitrate-responsive cis element and N-terminal region is the transcriptional activation domain responsible for nitrate activation. Our previous study showed that nitrate-regulated transcriptional response (primary nitrate response) is concentration dependent, and two phases of the response, the high-affinity phase and low-affinity phase, are differentially regulated. In this study, we would like to elucidate the role of NLP7 in the two-phase response. We used low (200mM) and high (25mM) concentration of nitrate to induce the expression of the nitrate-related genes in nlp7 mutant and wild type, and found out that different genes showed different expression defects in nlp7 mutant. While the expression of NRT1.1 (dual affinity nitrate transporter and transceptor) and NIR (nitrite reductase) show little or no defect in nlp7 mutant, fold changes of NIA (nitrate reductase) elicited by both high and low nitrate are significantly reduced in nlp7 mutant. In addition, we also examine the expression of several nitrate-inducible transcription factors (TF), including NLP1 and NLP3. Expression of three transcription factors including NLP1 is reduced in nlp7 mutant only found at high nitrate. These results indicated that nitrate regulated genes can be classified into three groups: NLP7 unrelated group (NRT1.1, NIR), NLP7-regulated concentration-dependent gene (TFs), and NLP-regulated concentration-independent group (NIA). To further elucidate how NLP7 participate in the primary nitrate responses, the interaction between NLP7 and other signaling components in the pathway is analyzed by yeast two-hybrid assay. Future study is in progress to illustrate how the dynamic interaction between NLP and the signaling components could trigger concentration dependent and gene specific responses of transcriptional regulation.

Keywords: nitrate, primary nitrate response, NLP7, NRT1.1

Poster #441. Regulation of stomatal development by antagonistic EPF peptides in Arabidopsis (Submission 401)
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Proper density and distribution of stomata are critical for efficient gas exchange between a plant and the atmosphere. Several EPIDERMAL PATTERNING FACTOR (EPF)/EPF-LIKE (EPFL)-family members of small cysteine-rich peptides have been discovered as important signaling molecules controlling stomatal patterning and differentiation in Arabidopsis. EPF1 and EPF2, emitted from stomatal precursor cells, inhibit neighboring cells from adopting stomatal-lineage cell fate. STOMAGEN, on the other hand, is expressed in the underlying mesophyll tissues and promotes stomatal differentiation in the epidermis. Interestingly, STOMAGEN appears to antagonize EPF1 and EPF2 function in controlling stomatal patterning, but requires the same receptor component, TOO MANY MOUTHS (TMM). Therefore, a next important question is how these contrasting peptides from the same family can produce opposite effects on stomatal development using the shared receptor component. Our group has recently demonstrated that TMM, which has no cytoplasmic signal transducing domain interact with ERECTA-family receptor kinases (RKs) in vivo and that perception of EPF1/2 by ERECTA-family RKs is critical for proper stomatal patterning. We took genetic and biochemical approaches to test if ERECTA-family RKs, known receptors for EPF1 and EPF2 peptides, also acts as the receptors for STOMAGEN during stomatal development. Furthermore, using recombinant, bioactive EPF peptides, we are currently investigating the activation patterns of ERECTA-family RK signaling by these contrasting peptides, EPF1/2 and STOMAGEN, in order to determine the molecular mechanisms of how different peptide signals are correctly interpreted using
the same receptor component(s) to control developmental responses. Our studies will provide for the first time new insight into how peptide signals may act antagonistically on the same receptor(s) to fine-tune stomatal production during development in plants.

Keywords: peptide signaling, stomatal development, receptor kinase

**Poster #442. Identification of an E3 ligase regulating the stability of BIN2 (Submission 405)**
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BIN2 is a GLYCOGEN SYNTHASE KINASE3ss (GSK3ss)-like kinase that acts as a negative regulator in signal transduction pathway of plant steroid hormone, Brassinosteroid (BR). BRs control diverse range of physiological processes including cell division, cell elongation, regulation of floral pattern, and stress responses. BIN2 phosphorylates and inactivates BR-specific transcriptional factors, BZR1 and BES1, and thus is considered an important factor in BR signaling. It is yet to be discovered how the activity of BIN2 is regulated in plants. So far, it was reported that BIN2 is regulated by ubiquitin/proteasome-mediated degradation, but still is necessary to identify the participating E3 ligases that may ubiquitinate BIN2. To this end, we took advantage of genetics and proteomics approaches. First, we made transgenic lines harboring loss-of-function and gain-of-function constructs where BIN2 is fused with TAP or HA epitopes. Transformants exhibited both semi-dwarf and severe dwarf phenotypes, indicative of successful expression of BIN2. To identify the BIN2 interacting proteins, we used different BIN2 transgenic lines and did immunoprecipitation followed by MALDI-TOF MS/MS. Second, we analyzed the gene expression at transcription level before and after BR treatment by using microarray data available in public database. By this approach we obtained a list of candidates of E3 ligases that are co-regulated with BIN2. Third, we’re currently testing whether these genes interact with BIN2 or not by using proteomic techniques like bimolecular fluorescence complementation assay and yeast two-hybrid assay. Through the application of these multiple approaches; genetic, proteomic approaches, we may be able to find BIN2 interacting proteins including E3 ligases responsible for BR signaling.

*Yu Jeong Jeong and Sliki Park are contributed equally to this work.

Keywords: BIN2, E3 ligase, ubiquitin proteasome mediated degradation, BR signaling

**Poster #443. Identification of an E3 ligase regulating the stability of BIN2 (Submission 406)**
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BIN2 is a GLYCOGEN SYNTHASE KINASE3ss (GSK3ss)-like kinase that acts as a negative regulator in signal transduction pathway of plant steroid hormone, Brassinosteroid (BR). BRs control diverse range of physiological processes including cell division, cell elongation, regulation of floral pattern, and stress responses. BIN2 phosphorylates and inactivates BR-specific transcriptional factors, BZR1 and BES1, and thus is considered an important factor in BR signaling. It is yet to be discovered how the activity of BIN2 is regulated in plants. So far, it was reported that BIN2 is regulated by ubiquitin/proteasome-mediated degradation, but still is necessary to identify the participating E3 ligases that may ubiquitinate BIN2. To this end, we took advantage of genetics and proteomics approaches. First, we made transgenic lines harboring loss-of-function and gain-of-function constructs where BIN2 is fused with TAP or HA epitopes. Transformants exhibited both semi-dwarf and severe dwarf phenotypes, indicative of successful expression of BIN2. To identify the BIN2 interacting proteins, we used different BIN2 transgenic lines and did immunoprecipitation followed by MALDI-TOF MS/MS. Second, we analyzed the gene expression at transcription level before and after BR treatment by using microarray data available in public database. By this approach we obtained a list of candidates of E3 ligases that are co-regulated with BIN2. Third, we’re currently testing whether these genes interact with BIN2 or not by using proteomic techniques like bimolecular fluorescence complementation assay and yeast two-hybrid assay. Through the application of these multiple approaches; genetic, proteomic approaches, we may be able to find BIN2 interacting proteins including E3 ligases responsible for BR signaling.

*Sliki Park and Yu Jeong Jeong are contributed equally to this work.
Keywords: BIN2, E3 ligase, ubiquitin proteasome mediated degradation, BR signaling

Poster #444. The role of protein phosphatase 2A in Arabidopsis development (Submission 407)
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Protein phosphatase 2A (PP2A) accounts for a large part of the serine/threonine phosphatase activity in the eukaryotic cell. PP2A is a heterotrimeric enzyme consisting of a scaffold A subunit, a regulatory subunit (B, B', and B''), and a catalytic C subunit. PP2A-C catalytic subunits are well conserved in eukaryotes. Arabidopsis has five PP2A-C genes that can be grouped in subfamily I (PP2A-C1, -C2, and -C5) and subfamily II (PP2A-C3 and -C4). Sequence similarity is over 95% for genes within subfamilies, and 80% between both subfamilies, suggesting a large degree of functional redundancy. We have used T-DNA mutant lines in all five PP2A-C genes to study the role of PP2A in Arabidopsis development. The combinations of double mutants reveal the involvement of PP2A in different developmental programmes, strongly suggesting that PP2A-C genes indeed fulfil partially overlapping functions. We have shown that disruption of subfamily II genes leads to abnormal embryo development. Our analysis has shown that polar localisation of PIN auxin transporters is altered in the double pp2a-c3 pp2a-c4 mutant. The collection of double mutant is also being used to study the dual involvement of PP2A in brassinosteroid signalling. The phenotypes observed in double mutants, but not in the single mutant parental lines, may reflect that the missing PP2A-C isoforms act on a common phosphorylated protein target. We are therefore analysing the expression patterns of PP2A-C genes aiming to establish their cell specific expression.

Keywords: protein phosphatase, developmental mutants, PIN auxin transporter, brassinosteroid signalling

Poster #445. The ribosomal protein S6 is involved in regulation of rRNA synthesis by possible chromatin modification in Arabidopsis (Submission 409)
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TOR (Target Of Rapamycin) kinase pathway regulates various biological processes including transcription of rRNA. The ribosomal protein S6 (RPS6) is one of the well-known downstream components of the TOR pathway. Ribosomal proteins have been known to have diverse functions in regulating cellular metabolism as well as protein synthesis. We report that RPS6 may have a novel function via interaction with the histone deacetylase 2B (AtHD2B), a member of the plant-specific histone deacetylase HD2 family. RPS6 and AtHD2B were localized to the nucleolus. Co-expression of RPS6 and AtHD2B caused a change in the location of both RPS6 and AtHD2B to one or several nucleolar spots. ChIP analysis suggests that RPS6 directly interacts with the rRNA gene promoter. Protoplasts overexpressing both AtHD2B and RPS6 exhibited down-regulation of pre-18S rRNA synthesis with concomitant decrease in some of the ribosomal proteins transcription, suggesting their direct role in ribosome biogenesis and plant development. This is consistent with the mutation in rps6b that results in reduction in 18S rRNA transcription and decreased root growth. We propose that the interaction between RPS6 and AtHD2B brings about a change in the chromatin structure of rDNA and thus plays an important role in linking TOR signaling to rDNA transcription and ribosome biogenesis in plants.

Keywords: RPS6, rRNA transcription, AtHD2B

Poster #446. Investigation of genetic interactions between DET1 and genes involved in ABA signal transduction in Arabidopsis thaliana (Submission 427)
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Plant development is regulated by both extrinsic and intrinsic factors. Seed germination is regulated positively by light and negatively by the dormancy-promoting phytohormone abscisic acid (ABA). It has been shown that DET1 is a negative regulator of light development and loss of function mutants (det1) have pleiotropic defects in expression of light-regulated genes in Arabidopsis thaliana. In contrast, the bZIP transcription factor LONG
HYOCOTYL 5 (HY5) is a positive regulator of light signaling and was identified as an integrator of light and hormone signaling pathways. HY5 positively regulates ABA signaling by promoting the expression of ABA INSENSITIVE 5 (ABI5), which is a dormancy promoting transcription factor in seeds. Here we show that light signaling mutant det1 is sensitive to ABA. In order to investigate the genetic interactions of HY5, ABI5 and DET1 we generated det1 hy5 and det1 abi5 double mutants. By analyzing det1 hy5 double mutants we found that ABA-sensitive germination of det1 require HY5. We show that ABA-sensitive germination of det1 also requires ABI5. DET1 forms a complex with DDB1 (DAMAGED DNA BINDING protein 1). Another DDB1 complex containing DWA1 and 2 (DWD hypersensitive to ABA 1 and 2) has also been shown to negatively regulate ABA response. Thus, we also investigated the det1 dwa1 and det1 dwa2 double mutants and we saw variable rescue of the det1 ABA sensitive germination phenotype. We also looked at adult water loss in det1 mutants and the double mutants and our data shows that the det1 rapid water loss phenotype is independent of any of the intermediate genes. The findings of this research provide insight into interactions between ABA and light signaling in Arabidopsis det1 mutants.

Keywords: Seed germination, ABA signaling, genetic interactions

Poster #447. Reduction in mitochondrial ATP affects a signaling pathway, including SnRK1 and SOG1, to control the Arabidopsis thaliana development (Submission 457)
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It is poorly understood how plants control their growth by cell division, elongation, and differentiation. We have characterized a seedling lethal mutant, segregation distortion 3 (sd3), which was isolated from RIKEN Ds insertional mutant collection. sd3 had short hypocotyl with reduced polyploidy levels, and showed a very dwarf phenotype when grown both in the light and dark conditions. The SD3 gene responsible to this mutation encoded a protein showing high similarity to a yeast translocase on the inner mitochondrial membrane 21 (TIM21), a component of the TIM23 complex. SD3 protein fused to GFP was localized in the mitochondria. SD3 over-expression plants accumulated amount of intracellular ATP, whereas sd3 showed a significant reduction of the intracellular ATP content, suggesting that SD3 expression may concern with the intracellular ATP production. Treatment with antimycin A, a mitochondrial electron transport inhibitor, resulted in formation of short hypocotyls along with decrease of polyploidy and reduced amount of intracellular ATP, which are similar to the sd3 phenotypes. These results suggested that length of hypocotyl is strongly affected by the amount of intracellular ATP produced in mitochondria. Our genetic interaction studies showed that the phenotype of sd3, short hypocotyl length and reduced polyploidy, was partially restored by introduction of each mutation of kin10/kin11, a subunit of Snf1-related kinase 1 (SnRK1) protein kinase complex, and suppressor of gamma response 1 (sog1). However, neither mutation restored the amount of intracellular ATP in sd3. In mammalian cells, AMPK is a paralogue of KIN10/KIN11, and directly interacts with p53. In plant cells, it has reported that SOG1 has a similar function to p53. We found SnRK1 interacted with SOG1. We suggest that there may be a novel regulatory mechanism involving SnRK1 and SOG1 in plants, which is involved in hypocotyl elongation.

Keywords: mitochondria, ATP, SnRK1, SOG1

Poster #448. Arabidopsis cluster B PP2Cs: their diversification and specificity in regulating MAPK pathways (Submission 459)
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Arabidopsis protein phosphatases 2C (PP2Cs) represent the biggest protein phosphatase family identified in eukaryotes. Up to ~80 PP2C genes have been found in model plant Arabidopsis, however their functions are less known. Previously, we have characterized 4 AP2Cs (from cluster B of Arabidopsis PP2C family) as MAPK
Calcium ions are important signaling molecules in environmental and hormonal responses of plant cells. Many biotic and abiotic stresses evoke a stress-specific Ca2+ signals in the cytoplasm, which act as a messenger in transducing external signals. Furthermore, recent studies have demonstrated that chloroplasts and mitochondria also generate a transient Ca2+ signals in response to environmental and biotic stresses, suggesting that these organelles are involved in the modulation of the spatio-temporal patterns of Ca2+ signals. In chloroplasts, thylakoid membrane-localized Ca2+ binding protein CAS has been identified as a regulator of both cytoplasmic and stromal Ca2+ signaling. However, molecular mechanisms underlying mitochondrial Ca2+ regulation remains largely elusive. In this study, we identified two candidates for mitochondrial Ca2+ regulators, MSL1 and MICU1, in Arabidopsis thaliana.

MSL1 is a homologue of bacterial Mechanosensitive (MS) channel of small conductance (MscS). MscS has been implicated in the cellular osmoregulation as a sensor of osmotic pressure. It is inferred that MscS may also act as a gateway for external Ca2+ ions in bacteria. Arabidopsis have ten MscS like, MSL genes.; MSL2 and MSL3 are localized predominantly to chloroplast envelope and are required for osmoregulation of plastids, whereas MSL4-10 proteins are likely to be localized to the plasma and/or vacuolar membranes. We focused on another plant homolog of the MscS, MSL1 that contains a putative mitochondrial transit peptide. We found that GFP tagged MSL1 proteins are localized exclusively to mitochondria. Furthermore, an immunoblot assay revealed that MSL1-GFP exists in the membranes of mitochondria. These results suggest that MSL1 is a mitochondria-localized MS channel in plants. We also discussed the physiological function and expression of MSL1 in Arabidopsis.

MICU1 (mitochondrial calcium uptake 1) is a mitochondrial EF-hand protein that regulates MCU (mitochondrial Ca2+ uniporter) channel activity and is required for Ca2+ uptake in animal cells. We characterized AtMICU1, an Arabidopsis homolog of human MICU1. We found that AtMICU1 is also localized to mitochondria in plant cells. Overexpression of AtMICU1 induced dysfunction of mitochondria, suggesting its role in the regulation of mitochondrial functions. Further characterization of MSL1 and MICU1 will shed light on the role of mitochondrial Ca2+ regulation in plant cells.

Keywords: protein phosphatases, PP2C, MAPK phosphatases, cell signaling, protein localization, MAPK signaling specificity

Poster #449. Identification and characterization of Arabidopsis MSL1 and MICU1 as putative calcium regulators in mitochondria. (Submission 460)
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Calcium ions are important signaling molecules in environmental and hormonal responses of plant cells. Many biotic and abiotic stresses evoke a stress-specific Ca2+ signals in the cytoplasm, which act as a messenger in transducing external signals. Furthermore, recent studies have demonstrated that chloroplasts and mitochondria also generate a transient Ca2+ signals in response to environmental and biotic stresses, suggesting that these organelles are involved in the modulation of the spatio-temporal patterns of Ca2+ signals. In chloroplasts, thylakoid membrane-localized Ca2+ binding protein CAS has been identified as a regulator of both cytoplasmic and stromal Ca2+ signaling. However, molecular mechanisms underlying mitochondrial Ca2+ regulation remains largely elusive. In this study, we identified two candidates for mitochondrial Ca2+ regulators, MSL1 and MICU1, in Arabidopsis thaliana.
Keywords: Mitochondria, Ca2+, Mechanosensitive Ion channel

**Poster #450. Regulation of plant stem cell quiescence by a Brassinosteroid signalling module** (Submission 505)
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The quiescent center (QC) maintains the activity of the surrounding stem cells within the root stem cell niche, yet specific molecular players sustaining the low rate of QC cell division remain poorly unknown. Here, we identified a R2R3-MYB transcription factor, BRAVO (BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTRE), acting as cell-specific repressor of QC divisions in the primary root of Arabidopsis. Ectopic BRAVO expression restricts overall root growth and ceases root regeneration upon damage of the stem cells, demonstrating the role of BRAVO in countering Brassinosteroid (BR)-mediated cell division in the QC cells. BR-regulated transcription factor BES1 (BRI1-EMS SUPPRESSOR 1) directly repress and physically interacts with BRAVO in vivo, creating a bistable switch that modulates QC divisions at the root stem cell niche. Together, our results define a mechanism for BR-mediated regulation of stem cell quiescence in plants.

Keywords: quiescent center, brassinosteroid, root stem cell

**Cell Walls**

**Poster #451. Functional analysis of a KNAT7-BLH6 transcription factor complex involved in regulation of secondary cell wall biosynthesis in Arabidopsis thaliana** (Submission 68)
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The TALE homeodomain transcription factor KNOTTED ARABIDOPSIS THALIANA7 (KNAT7) has been implicated in several studies as part of a regulatory network governing the commitment to secondary cell wall biosynthesis, where it appears to at least partially play a negative regulatory role. TALE homeodomain proteins of the KNAT and BELL1-LIKE HOMEODOMAIN (BLH) families are known to form functionally diverse heterodimers. Here we report that BLH6 specifically interacts with KNAT7 to regulate secondary cell wall development in the Arabidopsis inflorescence stem. We show that, like KNAT7, BLH6 is a transcriptional repressor, and that BLH6-KNAT7 physical interaction enhances the repression activities of KNAT7 and BLH6 alone. The overlapping expression patterns of BLH6 and KNAT7 and the phenotypes of blh6, knat7, and blh6 knat7 loss of function mutants are consistent with the formation of a functional BLH6-KNAT7 heterodimer that represses commitment to secondary cell wall biosynthesis in interfascicular fibers. In contrast, an additive irx phenotype of the double mutant suggests that KNAT7 and BLH6 may independently regulate vessel cell wall biosynthesis. BLH6 and KNAT7 overexpression results in thinner interfascicular fiber secondary cell walls, and these phenotypes are dependent on the interacting partner. Over expression of BLH6 under the control of the 35S promoter results in pleiotropic developmental phenotypes that are, however, not dependent on KNAT7. A major impact of the loss of BLH6 and KNAT7 function is enhanced expression of the HD-ZIP transcription factor REVOLUTA/INTERFASCICULAR FIBERLESS1 (REV/IFL1) suggesting that a key function of the BLH6-KNAT7 heterodimer is to repress REV/IFL1 expression. In support of the hypothesis that REV is a direct target of BLH6, BLH6 binds to the promoter of REVOLUTA directly and represses its expression. Moreover, plants over expressing BLH6 under the control of the 35S promoter displays similar phenotypes as rev loss-of-function mutants.

Keywords: Secondary cell wall, Transcription factor, protein complex
**Poster #452. Characterization of MUM ENHANCER 4, a gene required for mucilage production in Arabidopsis thaliana** (Submission 98)

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The mucilage secretory cells (MSCs) of Arabidopsis thaliana undergo complex differentiation whereby cell growth is followed by the synthesis and secretion of pectinaceous mucilage. MSCs provide a model system to study cell wall biosynthesis, secretion, and modification, and how these processes affect cell morphology and tissue properties. A number of genes have been identified affecting mucilage secretory cell differentiation, including MUCILAGE-MODIFIED4 (MUM4). mum4 mutants produce a reduced amount of mucilage and cloning of MUM4 revealed that it encodes an UDP-L-rhamnose synthase that is developmentally upregulated to provide rhamnose for mucilage pectin synthesis. To identify additional genes acting in mucilage production, a screen for enhancers of the mum4 phenotype was performed. Double mutants between mum enhancer 4 (men4) and mum4 lack mucilage release and show significant reductions in mucilage compared to mum4. The mum enhancer 4 (men4) single mutant exhibits a 30% mucilage reduction compared to wild type seeds, and altered MSC morphology. Chemical and immunological analyses suggest a reduction in the mucilage pectins rhamnogalacturonan I and possibly homogalacturonan, while no changes in Golgi morphology were obvious in preliminary ultrastructural studies. To determine the role of MEN4 in mucilage regulation, biosynthesis, or secretion, we have identified a candidate gene via Next-Generation Mapping that suggests MEN4 may function as a novel transcriptional regulator. We are currently characterizing the regulatory scope of MEN4 via both targeted and global transcription analyses. Investigation of MEN4 will contribute to our understanding of the complex regulation of cell differentiation processes including cell wall production.

Keywords: mucilage, pectin, biosynthesis, regulation, enhancer

**Poster #453. Cellulose deposition-independent mechanism of hemicellulose accumulation during xylem vessel cell differentiation** (Submission 270)

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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 cells and metaxylem-type cells with the helical and pitted patterns of SCW thickenings, respectively. We have been studying a NAC domain transcription factor, VND7, which is regarded to be a master regulator for xylem vessel cell differentiation in Arabidopsis. Overexpression of VND7 ectopically induces transdifferentiation of various cells into vessel elements with helical SCWs like protoxylem vessels. Recently, we established a transgenic Arabidopsis line with the overexpression of VND7 fused with the VP16 transactivation domain and the glucocorticoid receptor (35S::VND7-VP16-GR plant), in which most cells transdifferentiate into the "protoxylem vessel-like cells" with helical SCWs in a synchronous manner by dexamethasone application. For better understanding of molecular mechanisms underlying the formation of SCWs in vessel cells, we performed a genetic screening of EMS-mutagenized 35S::VND7-VP16-GR by microscopic observation of SCWs after the induction of ectopic vessel cell differentiation. Through the screening, we successfully isolated one mutant (tentatively called Line 35) with highly disordered SCW formation in the ectopic vessel cells. Curiously, in Line 35, the fluorescence of WGA-alexa488 showed the helical pattern like protoxylem vessel cells, while cellulose accumulation lost such patterns and lignin deposition was not detected. The mutant also showed irx (irregular xylem) mutant-like phenotype and became dwarf under normal growth condition. Taken together with these results, we will discuss the formation of SCWs in vessel cells.

Keywords: xylem vessel, secondary cell wall, VND7
Consistent with this movement, the mucilage cellulose appeared to be coiled around the columella, unwinding and the column of the cell, parallel with the surface of the seed in a pattern similar to that of cortical microtubules. GFP fusions we found that the CESA proteins were oriented and moved unidirectionally around the cytoplasmic column. During seed coat development, mucilage is secreted to the specific regions of the apoplast. This directed secretion causes the formation of a doughnut-shaped mucilage pocket around a volcano-shaped cytoplasmic column. Since proper synthesis and deposition of cellulose seem to be important for establishing normal mucilage adherence and organisation we investigated how CESAs deposit cellulose in the mucilage pocket. Using CESA-GFP fusions we found that the CESA proteins were oriented and moved unidirectionally around the cytoplasmic column of the cell, parallel with the surface of the seed in a pattern similar to that of cortical microtubules. Consistent with this movement, the mucilage cellulose appeared to be coiled around the columella, unwinding...
Keywords: seed coat, cell wall, mucilage, cellulose, cellulose synthase, CESA, microtubules, pectin

Poster #456. Using functional genomics to characterize genes involved in secondary cell wall biosynthesis in poplar and Arabidopsis (Submission 394)
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The carbon sequestered in secondary cell walls (SCWs) of wood is a renewable resource that can help decrease our dependence on fossil fuels. However, efficient conversion of wood-based biomass for use as an alternative fuel source is limited by the presence of lignin as well as our current understanding of SCW biosynthesis. Many aspects surrounding this highly dynamic process remain unknown, including the physiological processes that contribute to lignin content variation in SCWs. A large-scale genome-wide association mapping study in poplar (Populus trichocarpa) identified 25 genes associated with lignin content variation (Porth et al., (2013) New Phytologist 200: 710-726), many of which were unexpected and not yet known to have a SCW-related function. Using this data set, we are testing the hypothesis that these genes influence lignin content by characterizing the biological functions of a subset of these genes using a functional genomics approach in both poplar and Arabidopsis (Arabidopsis thaliana). In silico gene expression data was analyzed to identify genes with xylem-specific expression. Phylogenetic analyses were used to determine gene family sizes in poplar, extent of evolutionary conservation amongst land plants, and putative orthologs in Arabidopsis. A histochemical screen was carried out using Arabidopsis over-expression and T-DNA insertion mutants to identify defects in the cellular architecture of mature inflorescence stems. Preliminary findings suggest that genes of broad functional classes identified by the association mapping study affect SCW structure. Studying different classes of genes involved in SCW biosynthesis and lignin content variation will contribute to our understanding of wood development and the optimization of woody species for use as a bioenergy feedstock.

Keywords: bioenergy, lignin, secondary cell wall

Poster #457. FEI receptor kinases regulate cell wall function in Arabidopsis (Submission 410)
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Unlike animals, plants lack support structures such as endo- or exoskeletons and their form is maintained by a system of primary and secondary cell walls. Growing tissues respond to endogenous and exogenous signals to alter the structure and properties of the cell wall. Therefore, despite its rigidity, the cell wall is a highly dynamic structure that is regulated by a sophisticated sensing and feedback system. We are only just beginning to understand the pathways regulating cell wall biosynthesis and the mechanisms underlying integrity-sensing. Several members of the Receptor-Like protein Kinases (RLKs) family in Arabidopsis have been implicated in cell wall growth regulations in different contexts. FEI1 and FEI2 are two leucine-rich repeat (LRR) RLKs that regulate cell wall function in Arabidopsis. Mutations in both genes disrupt cell wall synthesis, which leads to impaired cell elongation and a short, swollen root phenotype. Studies in our lab have shown that blocking ethylene biosynthesis reverts the phenotype of fei1;fei2; surprisingly though, inhibition of ethylene perception or blocking the last step in ethylene formation does not rescue the swelling of the fei1;fei2 root. Moreover, attenuation of the direct ethylene precursor, ACC, suppresses the mutant phenotype, suggesting a signaling role for ACC.

In order to determine how exactly the FEI proteins regulate cell wall function, we sought to identify novel components of the FEI pathway and have isolated suppressors of the fei1 fei2 phenotype. One of the suppressor genes, SHOU4, encodes a protein of yet undescribed function. Interestingly, shou4 also partially restores the short, swollen root of cellulose synthase 6 (procuste-1) mutant. Our studies show that SHOU4 is localized at the plasma membrane in the root apical meristem and may interact with cortical cytoskeleton. The ongoing research in our laboratory aims to uncover the signaling role of ACC in regulating cell wall formation and the nature of the FEI-SHOU4 interaction.

Keywords: Receptor-Like Kinases, cell wall signaling, ACC, phytohormones
Poster #458. Understanding Pectin Biosynthesis: Modifying Seed Coat Mucilage of Arabidopsis thaliana (Submission 424)
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Plant cell walls perform a number of vital functions including structural support and defensive against abiotic and biotic stresses. One of the key components of a plant cell wall is pectin, which is a complex polysaccharide involved in strengthening the cell wall and providing cell-to-cell adhesion. Despite its importance, surprisingly little is known about pectin structure-function relationships. In order to study pectin, our laboratory is using the Arabidopsis thaliana seed coat epidermal cells as a model because of their ability to synthesize, and deposit to the apoplast, copious amount of pectin-rich mucilage. This system has been instrumental in identifying genes involved in mucilage biosynthesis, secretion and regulation using forward genetics.

Another way that this system can be used is to express and secrete carbohydrate active catabolic enzymes to modify mucilage and subsequently examine how the modifications affect mucilage properties and function. In order to achieve this highly-targeted genetic manipulation, seed-coat specific promoters derived from the DIRIGENT PROTEIN1, PEROXIDASE36 and MUCILAGE-MODIFIED4 genes will be utilized. As a first step towards assaying the utility of these promoters to modify mucilage composition, I plan to use these promoters to drive expression of a gene, MUCILAGE-MODIFIED2 encoding a known mucilage catabolic enzyme and test the ability of the chimeric gene to complement the mum2 mutation. Subsequently, these promoters will be used to drive gene expression of carbohydrate active enzymes designed to actively modify mucilage composition exclusively within the upper epidermal layer of the seed coat.

Keywords: Cell wall, Pectin, Seed coat, Seed coat mucilage, Seed-coat specific promoters, DP1 (DIRIGENT PROTEIN1), MUM2 (MUCILAGE-MODIFIED2), MUM4 (MUCILAGE-MODIFIED4), PER36 (PEROXIDASE36), Carbohydrate active enzymes

Poster #459. Secretion of pectin and its modifying enzyme MUM2 in seed coat epidermal cells of Arabidopsis (Submission 454)
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Pectin is a major component of the plant primary cell wall, and can be modified by enzymes to allow for loosening or strengthening during growth or in response to changes in the environments or development. Both pectin and the enzymes that modify it are secreted through the Golgi to the apoplast via vesicles. Understanding how pectin is modified to change cell wall properties is an important goal of plant cell research.

During differentiation, Arabidopsis seed coat epidemal cells make large amounts of seed mucilage which is deposited in the apoplast in a polar manner in large apoplastic pockets on the outer surface of the cell. The mucilage is made up primarily of pectin. Our laboratory uses this cell type as a model to study pectin synthesis and function. One protein secreted by the seed coat epidermal cells is MUCILAGE MODIFIED 2 (MUM2) a b-galactosidase that is needed to modify the physio-chemical properties of mucilage pectin. In an attempt to better understand MUM2 function, we are studying the temporal and spatial manner by which MUM2 is secreted during seed coat differentiation relative to its substrate pectin. To do this an engineered version of MUM2 fused to a yellow fluorescent protein (YFP) was constructed and shown to complement the mum2 mutant phenotype. The MUM2-YFP was found to be deposited in the mucilage pocket from 6 DPA to 8 DPA, coincident both spatially and temporarilly with its mucilage substrate. The signal of MUM2-YFP in the mucilage pocket abruptly disappears at 9 DPA, implying that MUM2 is degraded. The targeting of MUM2-YFP to the mucilage pocket suggests that it is secreted in a polar manner. To determine whether this pattern of accumulation is characteristic of all proteins secreted at this time by this cell type or is dependent on the sequence of specific target proteins like MUM2, we have expressed both MUM2-YFP and sec-YFP under the control of a promoter that targets both to the seed coat epidermis during mucilage synthesis. Our progress toward answering this question will be presented.

Keywords: seed coat, cell wall, pectin, pectin-modifying enzyme, secretion
Poster #460. Diurnal regulation of transcript levels of secondary cell wall biosynthetic genes in Arabidopsis thaliana and physiological significance of the KNAT7 repressor protein in these lignified tissues. (Submission 483)
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Lignified secondary cell walls in plants, made up of cellulose, hemicellulose and lignin, represent a large sink tissue for global fixed carbon reserves. Synthesis of these walls is an energetically expensive process for the plant and is largely irreversible; hence it follows that spatial-temporal control of secondary cell wall biosynthesis must be closely co-ordinated with carbon metabolism. A hierarchical network of transcription factors, including several MYB and NAC domain containing proteins, play an important role in controlling wall formation by the mechanism of mostly, transcriptional activation. A secondary-wall related protein KNAT7, on the other hand, acts as a repressor of transcription, in a plant protoplast system; corresponding to which, an Arabidopsis thaliana KNAT7 gene knock-out mutant exhibits defective secondary cell wall phenotypes and an increased lignin fraction in extracted wall biomass.

As part of my Masters’ thesis I hypothesise that KNAT7 functions in a negative regulatory loop for resource allocation to secondary cell wall biosynthesis. I have identified that transcript accumulation levels of my selected set of secondary cell wall biosynthetic genes, in mature wild type Col-0 stems, exhibits a characteristic pattern of upregulation and downregulation over a daily light-dark cycle. I am now investigating transcript accumulation patterns in stems of the KNAT7 gene knockout mutant. An altered pattern in the mutant would suggest a function for KNAT7’s transcriptional repression in diurnal regulation of secondary cell wall biosynthesis.

Keywords: diurnal, cell wall, lignin, transcription, genetic repression, KNAT7, irx

Gene Regulation

Poster #461. A comprehensive study of the MYB-bHLH-WDR transcriptional networks that control flavonoid biosynthesis (Submission 109)
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The combinatorial control of structural gene expression is a key feature of the spatiotemporal pattern of flavonoid accumulation in plants. In Arabidopsis thaliana, the regulation of anthocyanins and proanthocyanidins (PAs) pigmentation relies on the transcriptional activity of R2R3-MYB and bHLH proteins that form ternary complexes ("MBW") with TTG1 (WD-Repeats). The determination of the nature and spatiotemporal activity and the identification of the direct targets of these MBW complexes were essential steps towards a comprehensive understanding of the transcriptional mechanisms that control flavonoid biosynthesis. TT8/bHLH042 is a key regulator of anthocyanin and PA biosynthesis in Arabidopsis. Functional dissection of the TT8 promoter revealed its modular structure. Two modules were found to specifically drive TT8 promoter activity in PA- and anthocyanin-accumulating cells, by differentially integrating the signals issued from different regulators, in a spatiotemporal manner. Interestingly, this regulation involves at least six different MBW complexes. Moreover, the results presented suggest that some putative new regulators remain to be discovered. In addition, we have demonstrated that DFR, LDOX, BAN, TT19, TT12 and AHA10 are direct targets of the MBW complexes (and excluded CHS, CHI, F3H, F3'H, TT10 and TT15). Interestingly, although the TT2/MYB123-TT8-TTG1 complex plays the major role in developing seeds, three additional MBW complexes involving MYB5, GL3 and EGL3 were also shown to be involved, in a tissue-specific manner. The use of the newly developed fast and sensitive transient expression system that relies on the moss Physcomitrella patens protoplasts has allowed to identify both specific cis-regulatory elements through which TT8 expression is regulated and the minimal promoter for each of the genes that are targeted by the MBW complexes. Altogether, by answering fundamental questions and by demonstrating or invalidating previously made hypotheses, we have provided new and comprehensive view of the regulatory mechanisms controlling PA and anthocyanin biosynthesis in Arabidopsis, as well as new clues and tools for further investigation this pathway in Arabidopsis and other plant species.
These results will be discussed with the aim to highlight the key questions that remain to be answered and the new perspectives that should be given to this work.

Keywords: Arabidopsis, Flavonoid, Regulation, MYB-bHLH-WDR, cis-element

Poster #462. INVESTIGATING THE REGULATION OF CUTICULAR WAX BIOSYNTHESIS THROUGH THE CONTROL OF EXPRESSION OF ECERIFERUM 3 IN ARABIDOPSIS THALIANA (Submission 227)
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Cuticular wax is a key component of the plant cuticle, which covers the outer surface of all the primary aerial organs of plants, and serves as a barrier that protects plants from water loss, UV-light, pathogens and insects. Mutants that are defective in the biosynthesis of cuticular wax and/or regulation of cuticular wax production, such as cereriferum (cer) mutants of Arabidopsis thaliana, can provide us with valuable information about these processes. The isolation and characterization of the cer3 mutant demonstrated that ECERIFERUM3 (CER3) is a key wax biosynthetic gene, and permitted its isolation. To understand the regulation of the CER3 expression during cuticular wax biosynthesis in Arabidopsis stems, we have identified several transcription factors that directly bind to the 5' promoter region of CER3 using yeast one-hybrid screening, and are currently investigating their role in wax deposition. In addition, we have identified a wax-deficient mutant with a similar wax composition as the cer3, and confirmed that this novel mutant, designated cer30, is not allelic with cer3. Expression analysis revealed that the steady state transcript levels of CER3 in the cer30 mutant are reduced, suggesting that the CER30 protein may play a role in the CER3 expression. We will discuss our current understanding of the role of the identified transcription factors and the CER30 protein in controlling CER3 transcription during cuticular wax biosynthesis.

Keywords: cuticular wax, gene regulation, wax biosynthesis

Poster #463. Arabidopsis dehydration-responsive element binding factor, DREB2C, modulates ABA biosynthesis during germination (Submission 284)
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Plant dehydration-responsive element binding factors (DREBs) are transcriptional regulators of the APETELA2/Ethylene Responsive element-binding Factor (AP2/ERF) family that are control expression of abiotic stress-related genes. We show here that under conditions of mild heat stress, constitutive overexpression seeds of transgenic DREB2C overexpression Arabidopsis exhibit delayed germination and increased abscisic acid (ABA) content compared to untransformed control. Treatment with fluridone, an inhibitor of the abscisic acid (ABA) biosynthesis abrogated these effects. Expression of an ABA biosynthesis-related gene, 9-cis-epoxycarotenoid dioxygenase 9 (NCED9) was up-regulated in the DREB2C overexpression lines compared to wild type. DREB2C was able to trans-activate expression of NCED9 in Arabidopsis leaf protoplasts in vitro. Direct and specific binding of DREB2C to a complete DRE on the NCED9 promoter was observed in electrophoretic mobility shift assays. Exogenous ABA treatment induced DREB2C expression in germinating seeds of untransformed wild type. Vegetative growth of transgenic DREB2C overexpression lines was more strongly inhibited by exogenous ABA compared to untransformed wild type. These results suggest that DREB2C is a stress- and ABA-inducible gene that acts as a positive regulator of ABA biosynthesis in germinating seeds through activating NCED9 expression.

Keywords: Heat stress, Transcription factor, Transgenic plant

Poster #464. Ectopic expression of a cold-regulated 15A (COR15A) gene improves salt stress tolerance in Arabidopsis (Submission 287)
Chieun Song¹, Jihyun Je¹, Chae Oh Lim¹
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Cold acclimation in plants is associated with the expression of COR (cold-regulated) genes that encode a small, plastid-targeted polypeptide. It has been widely speculated that products of these genes might have roles in freezing tolerance. We report here that constitutive overexpression of COR15A from the cauliflower mosaic virus (CaMV) 35S promoter led to enhanced salt tolerance in transgenic Arabidopsis that was characterized by higher viability in plants grown in NaCl-treated pots. Promoter transactivation assays and electrophoretic mobility-shift assay showed that DEHYDRATION-RESPONSIVE ELEMENT BINDING FACTOR 2C (DREB2C) interacts directly with the three DREs in the COR15A promoter, both in vivo and in vitro. Transgenic Arabidopsis constitutively overexpressing DREB2C from the CaMV35S promoter exhibited greater NaCl tolerance than the untransformed wild-type. Taken together, the data suggest that DREB2C functions as transcriptional activator that promotes NaCl tolerance, in part through upregulation of the cold stress-responsive gene COR15A.

Keywords: NaCl stress, Transgenic plant, Transcriptional activator

Poster #465. The roles of iron-responsive transcription factors in gene regulatory networks involved in key processes of iron homeostasis. (Submission 293)
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Plants underpin almost all food chains, are vital to materials production, and sustain our atmosphere. However one-third of the earth’s soil is calcareous, having a pH higher than 7, which prevents Fe(III) from dissolving into usable Fe(II). Iron is an essential plant micronutrient required for growth and development. Since excess iron can be toxic, iron sensing, chelation, acquisition, signaling, transport, and storage are tightly regulated. Current microarray datasets collectively show that there are 147 iron-responsive transcription factors. Yet, little is known about their function in iron homeostasis. To address this, we are using a systems biology approach to understand the gene regulatory networks controlled by iron homeostasis transcription factors. Additionally, we are using scanning fluorescence correlation spectroscopy microscopy with transgenic lines expressing fluorescently tagged proteins to examine intra- and inter-cellular protein movement and interactions associated with the responses to iron deprivation. Using these approaches with standard molecular methodologies will give us insight into whether movement and dynamic interactions are essential to the functions of these iron homeostasis transcription factors and shed new light on how these proteins control regulatory networks involved in root response to iron deprivation.

Keywords: Abiotic Stress, Gene Regulatory Network, Scanning Fluorescence Correlation Spectroscopy

Poster #466. High Throughput Quantification of Phytohormones During Pseudomonas syringae p.v. tomato DC3000 - Arabidopsis Interactions (Submission 304)
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Plant hormones play an essential role in regulating plant development and responses to the environment, including response to pathogens. Recent research has uncovered a dynamic interplay of synergistic and antagonistic relationships between hormone signaling pathways during plant-pathogen interactions, many of which have not been fully investigated. With developments in high throughput molecular techniques and computational biology we now have the tools to be able to investigate these interactions at a level not previously possible. We are employing transcriptomics and phytohormone profiling to monitor global gene expression and the levels of 8 major plant hormones (abscisic acid, auxin, brassinosteroids, cytokinin, ethylene, gibberellic acid, jasmonic acid and salicylic acid) during the Arabidopsis immune response to the bacterial pathogen Pseudomonas syringae p.v. tomato DC3000. Using this pathosystem, we will explore compatible versus incompatible interactions as well as the transition from biotrophic to necrotrophic phases of infection. By studying patterns of global hormone-regulated gene expression in relation to endogenous hormone levels, we hope to reveal emergent properties of hormone signaling in response to pathogens. Data from this investigation will be combined with publicly available data to create a computational model describing the relationships between hormone signaling and defense pathways. Understanding these relationships will serve to strengthen our knowledge of plant immunity and potentially lead to novel methods of plant breeding through predictive modeling.
Keywords: Systems Biology, Plant Immunity, Metabolomics, Transcriptomics

**Poster #467. Identification of the NF-Y trimer including NF-YC10, which interacts with Arabidopsis DREB2A and regulates the stress specific expression of DREB2A target genes** (Submission 309)
Hikaru Sato¹, Junya Mizoi¹, Hidenori Tanaka¹, Kyonosin Maruyama², Feng Qin², Yuriko Osakabe², Kazuo Shinozaki³, Kazuko Yamaguchi-Shinozaki¹
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Appropriate gene expression in response to changes of the environmental conditions is necessary for plants to survive. Arabidopsis DREB2A is an important transcription factor that regulates dehydration and heat stress-inducible genes. The DREB2A protein is unstable and degraded by the ubiquitin-proteasome system under non-stress conditions, but under dehydration or heat stress conditions, this protein is stabilized and induces the expression of dehydration or heat stress-inducible target genes. Previous studies revealed that the "negative regulatory domain (NRD)" composed of 30 amino acids contributes to the degradation of DREB2A. The DREB2A protein lacking the NRD shows the increased stability and induces the expression of both dehydration and heat stress-inducible target genes even under non-stress conditions. These data suggest that DREB2A requires posttranslational regulation in response to stress signals for the protein stabilization and selectivity of stress specific target gene inductions. However, detailed mechanisms in which DREB2A induces dehydration and heat stress response remain to be elucidated.

In this study, we identified NF-YC10, a member of the NF-YC family protein, as an interacting protein with DREB2A using yeast two-hybrid screening. The NF-Y transcriptional regulator is conserved among eukaryotes and forms a heterotrimer composed of NF-YA, NF-YB and NF-YC subunits to regulate the transcriptional activity of a transcription factor. Arabidopsis thaliana have 10 NF-YA, 13 NF-YB and 13 NF-YC genes. NF-YC10-overexpressing Arabidopsis showed significantly increased heat stress tolerance, while the drought stress tolerance was not significantly changed. Moreover, the analysis of gene expression patterns revealed that the expression levels of heat stress-inducible DREB2A target genes were enhanced, while those of dehydration stress-inducible genes were not affected in the NF-YC10-overexpressing plants. These results suggest that NF-YC10 is a positive regulator of DREB2A specifically under heat stress conditions. Now, we are trying to identify the NF-Y trimer that enhances the transcriptional activation of DREB2A through interaction assay between the NF-Y subunits and gene expression analysis of the NF-Y family genes under dehydration or heat stress conditions.

Keywords: Abiotic stress, Posttranslational regulation, Transcriptional complex, Drought, Heat

**Poster #468. Involvement of mRNA stability control in ABA-promoted responses** (Submission 332)
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Plants have developed a set of mechanisms that allow adaptation to adverse environmental conditions to maintain their energetic homeostasis, hence ensuring their development and propagation. Such adaptive responses rely in part on the abscisic acid (ABA) signaling pathway, one of the plant hormones involved in abiotic stress responses. Kinetics of ABA-promoted changes in gene expression reveal that while the mRNA levels of ABA receptors of the PYL family are promptly repressed upon ABA treatment, the expression of type 2C protein phosphatases (PP2Cs) and of SNF1-related protein kinases 2 (SnRK2s) go through an activation period and into a stabilization phase. The analysis of the levels of ABA-responsive transcripts, including several transcription factors, suggests the existence of three major classes, i.e. early-activated genes, late-activated genes, and genes whose expression does not vary after activation. In addition, the evaluation of the mRNA stability of these transcripts also indicates that ABA-promoted post-transcriptional regulations may account for at least part of these responses, most likely reflecting a fast response to stress. These later results lead to the question of which would be the subjacent mechanisms involved in such regulation.

Keywords: Abscisic Acid, Gene expression, Post-transcriptional regulation
Poster #469. AtGFA1 plays an essential role in pollen germination in Arabidopsis. (Submission 353)
Ngoc Trinh Nguyen¹, Vu Van Kien², Suk-Whan Hong³
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The hexosamine biosynthetic pathway (HBP) is an essential branch of glucose metabolism, providing uridine 5'-diphospho-N-acetyl-D-glucosamine (UDP-GlcNAc). The first and rate-limiting step of the HBP is mediated by glutamine:fructose-6-phosphate amidotransferase (GlcN-6-P synthase). This regulatory enzyme is encoded by a single-copy gene (AtGFA1, At3g24090) in Arabidopsis. Three T-DNA insertion Arabidopsis mutant lines within AtGFA1 were examined to determine the physiological importance for the HBP. The homozygous gfa1-1 mutant with T-DNA in the promoter was found to show significant decrease in the AtGFA1 transcript level, resulting in 30% of the wild-type GlcN-6-P content, yet had little effect on growth and development. However, no individuals homozygous for gfa1-2 and gfa1-3 were identified with T-DNA in exon and intron, respectively. Progeny from self-cross of their heterozygous plants showed a segregation ratio of 1:1 for wild-type and heterozygous mutant plants, indicating a gametophytic defect. Reciprocal crosses and segregation analyses showed no transmission of the gfa1-2 and gfa1-3 alleles through the male gametophyte, while female gametophytic transmission was reveal to be little affected. The male gametophytic defect was further supported by approximately 50% reduction of pollen germination in plants heterozygous for gfa1-2 and gfa1-3. Interestingly, a quantitative RT-PCR and histochemical staining analysis showed significantly high abundance of AtGFA1 transcripts in flower compared with other organs. Introduction of E.coli glmS gene, encoding GlcN-6-P synthase can complement the lethal phenotype of gfa1-2. These findings suggest that a proper supply of glucosamine would be essential for pollen tube germination and growth during fertilization in Arabidopsis.

Keywords: Glucosamine, The hexosamine synthetic pathway, Lethal mutation, Pollen development

Poster #470. Regulation of TALE gene expression in Arabidopsis thaliana (Submission 412)
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Homeodomain proteins are characterized by highly conserved DNA binding domain and they participate as transcription factors in the regulation of different developmental processes. Based on sequence homology, plant homeodomain proteins can be divided into several classes. KNOX and BEL families belong to a highly conserved supergroup of three-amino-acid-loop-extension (TALE) homeodomain proteins. The Arabidopsis genome contains eight KNOTTED1-like genes and thirteen BEL-like (BLH) genes, most of them in overlapping regions. The expression of most of them was confirmed also for root tissues, but the function of these genes in root development is not so clear. We have characterized expression pattern and hormonal regulation of chosen TALE genes especially in root tissues. Detailed analysis of auxin (IAA) influence on lateral root primordia initiation in seedlings with changed BP (BREVIPEDICELLUS) transcript level indicates participation of TALE proteins in auxin-mediated regulation of root branching.

Keywords: homeodomain, KNOX, BLH, expression, auxin, brevipedicellus, regulation

Poster #471. Whose transcript is it anyway? Global analysis of cytoplasmic mRNA decay rates. (Submission 445)
Malia J. Deshotel¹, Leslie E. Sieburth¹
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Cytoplasmic mRNA decay is an important component of the post-transcriptional regulation of gene expression and is essential for seedling development, but many aspects of the control of cellular RNA levels remain unknown. Cytoplasmic mRNA decay occurs through three main pathways: decapping followed by 5'-3' decay, 3'-5' decay through the exosome, and 3'-5' decay by the recently identified exoribonuclease SUPPRESSOR OF VARICOSE (SOV). Decay rate observations of a random sampling of mRNAs isolated from wild type seedlings and in mutants lacking decapping and SOV activity revealed some transcripts that were specific for a particular decay pathway, and other transcripts that were degraded promiscuously. These surprising results prompted us to ask how mRNAs are commonly chosen for specific decay pathways, and what mechanism is behind decay pathway choice. To address these questions, I am conducting global mRNA decay assays in four different genetic
backgrounds. I am using the wild type Col-0 accession, which is a natural SOV mutant, and transgenic Col-0 in which SOV activity has been restored. Additionally, I am using varicose (vcs) sov double mutants, which lack mRNA decapping and SOV activity, as well as vcs mutants with a functional SOV transgene. I am blocking transcription in early seedlings, collecting samples over increasing time points, and assessing transcript abundance using RNAseq. For every gene in each of the four genotypes, I am calculating mRNA decay kinetics. In this way, the comparison of mRNA decay rates should yield the contribution of decay machinery to regulating transcript levels. mRNAs that are known substrates of more specialized decay pathways such as NMD and small-RNA directed internal cleavage will be screened for separate analyses. This work will provide a genome-wide understanding of mRNA decay specificity, and also provide a launching point for the discovery of RNA characteristics that allow mRNA substrate recognition by these pathways.

Keywords: mRNA decay, mRNA decapping, post-transcriptional gene regulation, varicose, SOV

RNA Biology

Poster #472. RBP1, a novel plant specific mRNA-binding protein (Submission 298)
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Ribonucleoprotein complexes (RNPs) consisting of RNA-binding proteins (RBPs), mRNAs and/or non-coding regulatory RNAs have been shown to play a crucial role in the spatial and temporal regulation of mRNA translation in many model organisms. In plants, however, these processes are not well studied. More than 200 putative RBPs have been predicted, many of them are thought to participate in biological processes restricted to plants. Some of these plant-specific RBPs are involved in response to environmental stress or developmental processes. With our work, we would like to shed some light on the functions of ribonucleoprotein complexes in vegetative and reproductive development of the model plant Arabidopsis thaliana. For this purpose, we started to characterize the molecular functions of the novel plant specific mRNA-binding protein RBP1 and its homologues. RBP1 appears highly expressed in dividing cells such as meristematic tissues and during stomata development as well as in young developing tissues such as ovules and embryos. RBP1-GFP localizes to some extent in moving cytosolic globular particles and has a RNA binding capacity suggesting that it is part of higher order RNP complexes. We are currently searching for knock out or knock down mutants of RBP1 and its homologues to characterize the biological functions of this new protein family in plant development. The identification of interaction partners by in vivo pull down experiments and more detailed functional studies using, for example, protein subdomains will provide a closer look on protein function, RNA binding, and regulation of RBP1 and its homologues.

Keywords: translational control, RNP complex, meristem, RRM

Poster #473. dsRNA-cleaving activities of DCL3 and DCL4 are changed with depending on organs or developmental stages in Arabidopsis thaliana and Brassica rapa. (Submission 330)
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RNA interference (RNAi) is a regulation mechanism of gene expression by small RNAs, such as micro RNAs and small interfering RNAs (siRNAs). Dicer-like protein 3 (DCL3) and DCL4 make siRNAs of 24 nt and 21 nt, respectively, from double-stranded RNAs (dsRNAs) in Arabidopsis thaliana. Biochemical characterization of dsRNA-cleaving activities in crude extracts isolated from Arabidopsis seedlings demonstrates enzymatic properties of DCL3 and DCL4 [Fukudome et al, RNA 17.4 (2011): 750-760, Nagano et al, Nucleic acids research (2013): gkt1077]. RNAi is essential for plant development, but it remains unknown how DCL3 and DCL4 are involved in them.
In this study, we examined DCL3 and DCL4 activities of callus, flowers, siliques, leaves, petals, pollens and ovules of A. thaliana or B. rapa. The DCL3 activity was relatively more stable than the DCL4 activity in all the
tested organs; however, the DCL4 activity in callus, the dedifferentiated or un-differentiated organs, was higher than that in leaves, the differentiated organs. During hypocotyls dedifferentiation, i.e. induction of callus formaion, the DCL activities (especially DCL4 activity) were dependent on the concentration of auxin (2,4-D) and cytokinin (kinetin) contained in callus-inducing medium.

For detailed analysis of DCL activities in flower organs, we used Brassica rapa, one of relative species of A. thaliana, which has bigger flowers than A. thaliana. We detected the activities producing 21 and 24 nt RNAs from 50 nt dsRNAs in crude extracts of Brassica petals, pollens and ovules. By using anti-AtDCL3 and anti-AtDCL4 antibodies, it was shown that Brassica DCL3 and DCL4 produced 24 and 21 nt RNAs, respectively, from 50 nt dsRNAs. The Brassica DCL4 activity is higher in ovule than that in other organs including pollen. These results demonstrate that the DCL activities are differentially regulated in a developmental stage- and/or organ-dependent manner.

Keywords: DCL3, DCL4, callus, flower, Brassica rapa, pollen, ovule, siRNA, dsRNA

Poster #474. Nutritional deficiency stresses affect DCL3 and DCL4 activities in Arabidopsis thaliana. (Submission 351)
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Dicer-like 3 (DCL3) and Dicer-like 4 (DCL4) are involved in DNA methylation and defense against viral infection, respectively. dsRNA-cleaving activities of DCL3 and DCL4 are measured in crude extracts isolated from Arabidopsis seedlings by detecting 24 and 21 nt RNAs, respectively, from 32P-labeled 50 nt dsRNAs. In this study we measured DCL3 and DCL4 activities when a concentration of phosphate, nitrate, sulfate, acetate or metal ion (Mg, Mn, Ca) was changed in reaction mixtures. The results indicated that phosphate enhanced the DCL3 activity and reduced the DCL4 activity more effectively than other anions, and that the optimum concentration of Mg or Mn, which is essential for dsRNA-cleaving activity of Dicers, for DCL3 was lower than those for DCL4.

Furthermore, we examined DCL3 and DCL4 activities in crude extracts isolated from Arabidopsis seedling which were grown in nutritional deficient media. These results showed that the DCL3 and DCL4 activities were maintained during phosphate deficient treatment. The DCL4 activity detected from crude extracts was decreased when Arabidopsis seedlings were subjected to sulfur or nitrogen deficient treatment. However, the DCL4 activity was detected considerably when an immunoprecipitate purified by using an anti-DRB4 antibody was used as an enzyme fraction for Dicer assay. These results suggest that nitrogen or sulfur deficient stress may inhibit the DCL4 activity in seedlings.

Because the DCL4 activity is changed with nutritional deficiency treatment, we investigated how nutritional deficient stresses affect PTGS (post-transcriptional gene silencing) for which DCL4 is essential. The JAP3 plants, in which PTGS is artificially induced on the PDS (phytoene desaturase) gene, were grown under nutritional deficient condition, and areas of photobleaching by silencing of the PDS gene in leaves of JAP3 plants were observed. The results revealed that the phosphate deficient treatment may enhance PTGS.

Keywords: DCL3, DCL4, nutritional deficient stress, PTGS

Poster #475. The effect of dsRNA structures on RNA interference in Arabidopsis protoplasts (Submission 365)
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Regulating specific gene expressions is important for basic and applied science such as research tools, medical purposes, new bleeding technology of plants and so on. RNA silencing (RNA interference) is a mechanism which regulates specific gene expressions by mRNA degradation or DNA methylation. Small RNAs (20 - 28 bases) are produced from double-strand RNAs (dsRNAs) by Dicers. These small RNAs are used for a guide of mRNA degradation or DNA methylation. The small RNAs have almost the same sequence with target mRNAs or DNAs, which make it possible to regulate specific gene expressions. In Arabidopsis, there are four Dicer-like proteins
(DCL1 - 4), and each of them has a specific role in RNA interference. Our previous research revealed that DCL3 mainly cleaves short dsRNAs into 24 nt siRNAs and DCL4 mainly cleaves long dsRNAs into 21 nt siRNAs. We analyzed whether DCL3 and DCL4 preferentially cleave short and long dsRNAs, respectively, in protoplasts. We used many kinds of dsRNAs with different lengths, which were transcribed from three genes (Green Fluorescence Protein, Actin2 and Elongation Factor 1A). We transfected protoplast with these dsRNAs, and then measured the quantity of target mRNAs. We found that long dsRNAs of about 100 nt in length were more efficient for RNA silencing (probably by mRNA cleavage). Actually, 21 nt siRNAs were produced from transfected long dsRNAs of about 100 nt, but these 21 nt siRNAs were not detected in dcl4 protoplasts. These results suggest that DCL4 preferentially cleaves long dsRNAs into 21 nt siRNAs in protoplasts that function RNA silencing (probably by mRNA cleavage). We are now conducting further experiments to reveal the effect of dsRNA structures on a DCL3 function by using protoplasts.

Keywords: RNAi, RNA silencing, protoplast, Dicer, DCL, epigenetic, siRNA

Poster #476. Towards the identification and characterization of RNA-binding proteome in Arabidopsis thaliana (Submission 376)
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Understanding of the role of RNA-binding proteins (RBP) in plants is still in its infancy. RBPs are multifunctional cellular regulatory proteins critical for the genesis, processing, translocation, translation, modification and degradation of RNA. Some RBPs such as Puf proteins have essential roles in controlling gene expression at the post-transcriptional level by promoting RNA decay and repressing translation[1]. RBPs have also been shown to be critical in RNA mediated-gene regulation during development, hormone signaling and redox regulation in Arabidopsis. Although RBPs have been well characterized in animal and fungal systems little is known about their function in plants and their role at the systems level, in particular in plant stress remains elusive. To systemically identify RBPs of Arabidopsis cv. Col-0 cells in response to salt stress, an interactome capture technique that combines UV cross-linking of RBP to RNA in vivo, oligo(dT) capture and mass spectrometry was employed to firstly optimize the system[2]. Thus far, >250 candidate proteins were identified with this system, and about 56% of the proteins have not previously been annotated as RBPs nor do they appear to contain any known single or double stranded RBP motifs. Forty-five proteins including ARGONAUTE 3, multidrug resistance-associated protein 10, guanyl-nucleotide exchange factors, ARM repeat superfamily protein, disease resistance protein and RNA-binding KH domain-containing protein having K-homology domain were identified. Furthermore, two promoters, ARF-binding site and Z-box promoter motifs were enriched. Finally, gene ontology enrichment showed bias towards processes such as binding (44% of the identified proteins), cellular process (35%), catalytic activity (33%), metabolic process (31%) and response to stimulus (11%). The study confirms and adds putative RBPs to the atlas of plant RBPs providing an important background for our ongoing work on charactering RNA-RBP network in Arabidopsis during stress and salinity in particular.

Keywords: RNA-binding proteins, Arabidopsis thaliana, Post-transcriptional regulation, RNA, Mass spectrometry

Translational Biology and Non-Arabidopsis Systems

Poster #477. Small GTP binding protein, OsRab11 is involved in defense Signaling (Submission 22)
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Rab GTPases are a large family of proteins with a variety of regulatory functions in membrane traffic and development. Previously, we characterized OsRab11, which in concert with OsGAP1 and OsGDI3 regulates vesicular trafficking from the trans-Golgi network (TGN) to the plasma membrane or vacuole. To further elucidate the physiological function of OsRab11 in plants, we performed yeast two-hybrid screens using OsRab11 as bait.
OsOPR8 was isolated and shown to interact with OsRab11. A co-immunoprecipitation assay confirmed this interaction. The green fluorescent protein-OsOPR8 fusion product was targeted to the cytoplasm and peroxisomes of protoplasts from Arabidopsis thaliana. OsOPR8 exhibited NADPH-dependent reduction activity when 2-cyclohexen-1-one (CyHE) and 12-oxo-phytodienoic acid (OPDA) were supplied as possible substrates. Interestingly, NADPH oxidation by OsOPR8 was increased when wild-type OsRab11 or the constitutively active form of OsRab11 (Q78L) were included in the reaction mix, but not when the dominant negative form of OsRab11 (S28N) was included. OsRab11 was expressed broadly in plants and both OsRab11 and OsOPR8 were induced by jasmonic acid (JA) and elicitor treatments. Overexpressed OsRab11 transgenic plants showed resistance to pathogens through induced expression of JA-responsive genes. In conclusion, OsRab11 may be required for JA-mediated defense signaling by activating the reducing activity of OsOPR8.

Keywords: OsRab11, GTPase, trafficking

Poster #478. Deep Sequencing Analysis of Brassica napus Lines Selected for Low Respiration and High Yield (Submission 52)
Marina Byzova1, Cindy Martens2, Magdalena Woloszynska3, Tom Verwulgen3, Marc De Block2, Mieke Van Lijsebettens3
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Plants utilize sophisticated epigenetic regulatory mechanisms to coordinate changes in gene expression and thus, rapidly and reversibly to respond to the environment. Moreover, one of the most complex quantitative traits in plants, yield, has been demonstrated to possess an epigenetic component that correlates with energy homeostasis [1]. The respiration rate was used as a selection marker for recurrent artificial selection in isogenic Brassica napus doubled haploid Simon populations. Selected lines with a low respiration rate had an increased seed production while those with high respiration were characterized by a decreased seed yield. All selected lines were found to be genetically identical but epigenetically different. Epigenetic states as well as the agronomic and physiological characteristics of the lines were stably transmitted for over eight generations [2].

To provide more insight into regulatory and metabolic pathways associated with transcriptional and epigenetic states, we have performed RNA deep sequencing analyses on polysomal RNA of the selected low-respiration lines. To analyse expression data of tetraploid B. napus lines, a bioinformatics pipeline was used based on mapping reads to non-redundant coding DNA sequences of two closely related diploid Brassica species, Brassica oleracea and Brassica rapa. Differential expression testing was done using the R package EdgeR. In house developed bioinformatics tools as BINGO, PLAZA and CORNET were used for identification of key regulatory pathways and genes.


Keywords: Brassica napus, Epigenome, RNA-seq

Poster #479. IRES-mediated expression of an RNA silencing suppressor (Submission 306)
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The internal ribosome entry site (IRES) enables bicistronic expression of multiple genes from a single mRNA. IRES-mediated translation often results in much lower protein expression than translation of single-gene mRNAs. We first improved IRES-mediated gene expression using a transient expression system based on Nicotiana benthamiana. We demonstrated that the presence of two cassettes comprised of an IRES-mediated coding sequence results in higher expression than one cassette. This double IRES cassette system is expected to be useful for expressing a gene of interest bicistronically with other genes from one mRNA. Using the system, bicistronic expression of a reporter gene and two copies of an IRES-mediated RNA silencing suppressor gene protected the transcribed mRNA from siRNA-mediated degradation like several RNA viruses. Thus, we set up mRNA self-protection system from RNA silencing by IRES-mediated expression of viral RNA silencing suppressor.
Keywords: IRES, RNA silencing suppressor, non-Arabidopsis

Poster #480. QTL analysis of leaf morphological traits in Japanese traditional leafy vegetables, Mizuna and Mibuna (Submission 308)

Yaichi Kawakatsu¹, Kaori Kaminoyama¹, Kaori Igarashi², Hokuto Nakayama³, Nakao Kubo⁴, Kentaro Yano⁵, Seisuke Kimura¹

Mizuna and Mibuna (Brassica rapa var. nipposinica) are traditional leafy vegetables in Kyoto, Japan. Although Mizuna and Mibuna are closely related, their leaf shapes are greatly different. Mizuna has unique serrated leaf, whereas Mibuna has spathulate leaf. To identify genes that are responsible for producing their morphological diversity, we have performed genetic analysis.

To perform genetic analysis, we generated F1 and F2 populations by a cross between Mizuna and Mibuna. Leaf shapes of F1 plants showed intermediate phenotype, and F2 population did not show any typical discrete segregation. These results suggest that several loci control the leaf shape, and these loci could be detected by QTL analysis.

Transcriptome analysis of Mizuna and Mibuna was performed for developing CAPS markers, which are needed for QTL analysis. RNAseq analysis was performed for Mizuna and Mibuna, and 4286 SNPs were found. Based on this result, 131 markers were developed, and 83 CAPS markers were mapped on linkage map. For scoring traits in each individual plant, we measured three phenotypes, complexity of leaf shape, height of serration, and depth of lobe.

QTLs with a large effect on complexity of leaf shape were observed on chromosome 1, 6, 7, and 10. In the case of height of serration, QTLs with a large effect were found on chromosome 6 and 7. Two QTLs for depth of lobe were detected on chromosome 1 and 10.

Comparing these results, four QTLs for complexity of leaf shape were identical to the two QTLs for height of serration and the two QTLs for depth of lobe. These results suggest that the leaf shapes variation between Mizuna and Mibuna are controlled by these four QTLs.

One of the major QTLs for lobes was detected on chromosome 10. Synteny analysis revealed that homolog of GA20ox3 was located near this QTL. GA20ox is associated with gibberellin (GA) biosynthesis. GA promotes cell growth and leads to a simplification of the leaf shape. Actually, when GA was applied on Mizuna shoot, their lobes were disappeared and more simplified leaves were developed. GA20ox3 might be responsible for leaf shape variation between Mizuna and Mibuna.

Keywords: Brassica rapa, leaf shape, QTL analysis

Poster #481. Developmental and molecular mechanisms of the heterophylly in North American lake cress (Rorippa aquatica; Brassicaceae) (Submission 403)

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Plants can show differential leaf forms in response to environmental changes, and this phenomenon is called heterophylly. Although heterophylly is seen across the plants, the mechanisms are largely unknown. Here, we investigated the developmental mechanism underlying the heterophylly in Rorippa aquatica (Brassicaceae), also known as North American lake cress. R. aquatica develops pinnately compound leaves in submerged conditions, whereas it forms simple leaves with serrated or lobed margin in terrestrial conditions. The expression levels of KNOTTED1-LIKE HOMEOBOX (KNOX1) orthologs changed in response to the ambient temperature and under/above water condition as well as the accumulation level of gibberellin (GA), which is thought to be regulated by KNOX1. We further demonstrate that exogenous GA affects the complexity of the leaf shape. Moreover, RNA-seq analysis indicated that the heterophylly is induced by the changes in light intensity. These results suggest that switching of the KNOX-GA module is involved in the heterophylly in R. aquatica. The
mechanism responsible for morphological diversification among species may also be employed in developmental plasticity.

Keywords: gibberellin, Heterophylly, KNOX, phenotipic plasticity, Rorippa aquatica

Poster #482. Diversity of Camelina spp. (Submission 456)
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11 wild relatives of C. sativa has been historically known with the center of origin in Eurasia (Akeroyd 1993) while C. sativa, C. rumelica, C. microcarpa, C. hispida, and C. alyssum are mostly recognized. Chromosome counts include 2n = 12 for C. rumelica (Brooks 1985), 2n = 14 for C. hispida (Maassoumi 1980), and 2n = 40 for C. sativa, C microcarpa, and C. alyssum (Francis and Warwick 2009). Although 2n = 40 is the most common count for those three species, many different counts have been found in various regions, indicating that natural variation among populations is prevalent with different ploidy numbers or aneuploidy. For C. sativa with 2n = 40 (the most common in cultivars), the genetic study conducted by (Gehringer et al. 2006) reported disomic inheritance and multiple amplifications of Brassica-derived SSR markers, suggesting that it could be an allopolyploid. More recently, (Hutcheon et al. 2010) found that two important genes, Fatty Acid Desaturase 2 (FAD2) and Fatty Acid Elongase 1 (FAE1), involved in fatty acid metabolisms are existing in three copies in C. sativa, in contrast to existing in single copy in Arabidopsis, the closest relative, suggesting that the genome of C. sativa may be an allohexaploid. C. sativa genome project has been also initiated (http://www.camelinadb.ca/) and the project page provides blast search against 20 C. sativa pseudomolecules but no additional details (eg. sequences, annotations, and high-density genetic map) have been yet released. Genomics-assisted breeding may be facilitated with the release of the entire genome in the near future. In the current study, a total of 20 Camelina spp. (mostly C. sativa) accessions collected in different locations in Europe and showed different agronomic traits are sequenced with reduced representation library (RRL) using the Illumina's HiSeq technology. In turn, the sequences are aligned to Arabidopsis genome to understanding of Camelina spp.

Keywords: Camelina, HiSep, germplasm

Workshop Speakers

Talk #483. ABA signalling pathway influences direction of root tip growth under low water potentials (Submission 404)
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Drought represents a major abiotic stress reducing crop yields globally. Roots play a key role exploring the soil to acquire important resources such as water. Root growth and development is influenced by environmental signals such as water gradients. Hence, root tropic responses represent key adaptive traits. For example, hydrotropism guides root growth away from regions with low water potential, thereby providing an important survival mechanism under water limiting conditions. Recent research has revealed that hydrotropism depends on the phytohormone abscisic acid (ABA), but the molecular and cellular mechanism(s) of action remain unclear.

To study this response we have used two experimental systems. The first employs a water potential gradient on agar plates. Under these conditions, wild type roots change growth direction in order to avoid low water potentials, and the angle formed is used to measure the hydrotropic response. We have analysed the hydrotropic response of mutants affected in the ABA signalling pathway and some of them have altered root bending. The second approach analyses the hydrotropic response in soil using X-ray microtomography (MicroCT). This approach allows the non-invasive study of root adaptive responses in plants’ natural environment. Our results show that, in agreement with our previous results in agar plates, roots of wild type plants tend to avoid low water potentials. Several studies have reported that plant hormones control root growth by targeting specific cells in different tissues. To elucidate which tissues are the principal targets in ABA regulation of root growth transgenic lines expressing components of the ABA signalling pathway under the control of different tissue-specific promoters were generated. The expression of SnRK2.2 (one of the SnRK2 protein kinases participating in the ABA signalling
pathway) in cortical cells was able to restore ABA sensitive root growth in the double mutant snrk2.2snrk2.3. In addition, seedlings expressing SnRK2.2 in the double mutant background in the cortex showed similar hydrotropic response than wild type. We conclude that ABA regulates this important adaptive root growth response by targeting dividing cortical cells at the proximal end of the meristem.

Keywords: ABA, Hydrotropism, Root growth, MicroCT

Talk #484. Becoming and being a successful researcher at a PUI (Primarily Undergraduate Institution) (Submission 421) Pablo Jenik¹, Lynn Pillitteri²
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Primarily Undergraduate Institutions (PUIs) are Colleges and Universities that, as the name indicates, enroll mostly undergraduate (and in some cases Master) students, but have no Ph.D. programs. Although, these institutions are a relatively unexplored career alternative for people formed at research-intensive universities, they provide an exciting environment for those interested in combining research and teaching. Doing research at PUIs has its unique rewards and challenges. The workshop is targeted at postdoctoral fellows and graduate students interested in faculty positions at PUIs. This workshop is aimed at introducing faculty life at a PUI. Facilitators in the workshop are drawn from a range of PUI institutions. Three main topics will be covered.
- What is a PUI?
- How to be a successful researcher at a PUI.
- Applying for a job and preparing for an interview at a PUI.
Discussion of other topics and alternate questions from participants is encouraged.

Keywords: Career, PUI, Teaching

Talk #485. Beyond the chloroplast: linking retrograde signals to ABA signaling in guard cells and seeds (Submission 503) Wannarat Pornsiriwong¹, Gonzalo M. Estavillo¹, Kai Xun Chan¹, Peter A. Crisp¹, Su Yin Phua¹, Pip B. Wilson¹, Christopher I. Cazzonelli¹, Barry J. Pogson¹
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Chloroplast-nuclear retrograde signaling pathways have been viewed as a means for bi-lateral communication between organelles and nuclei, largely ignoring the potential for interaction with hormone signaling regulating plant function. Exogenous and endogenous manipulation of the retrograde signal, phosphoadenosine phosphate (PAP), enables ABA-mediated stomatal closure conferring drought tolerance in ABA insensitive 1 (abi1) and open stomata 1 (ost1) mutants; likely via a distinct, exoribonuclease (XRN)-mediated transcriptional pathway in guard cells. PAP also acts as an ABA agonist in seed germination independent of ABI1. Thus, retrograde signaling mediated by PAP engages ABA signaling, likely via a distinct pathway to the canonical ABI1/OST1, to regulate stomata closure and germination. This reveals a process by which aspects of organelle communication, hormonal signaling and physiological responses to environmental stimuli might be integrated.

Keywords: Retrograde sign, ABA, Guard cells

Late Poster Abstract
Poster #486. (Cell Biology) FOUR LIPS/MYB124 stabilises PIN3-mediated auxin transport to regulate lateral root initiation. Qian Chen. Department of Plant Systems Biology, VIB/Ghent University, Gent, Belgium
The complex architecture of plant roots arises from the formation of branches or lateral roots that are essential for plant function as well as global soil retention. The plant hormone auxin frequently plays a master role in organogenesis and morphogenesis (Vanneste and Friml, 2009), and is particularly important for lateral root development (Lavenus et al., 2013). Recently, auxin was also implicated in stomatal development and spacing (Le et al., 2014). Here, we show that the FOUR LIPS/MYB124 (FLP) transcription factor, ensuring a terminal division in the stomatal lineage (Lai et al., 2005), acts as a downstream effector of auxin signalling in developing lateral roots. We found that FLP regulates lateral root initiation through a direct control to the auxin-induced expression of the PIN3 auxin efflux transporter. The effect of FLP is to stabilise auxin-induced PIN3 expression via a coherent feed forward loop with the Auxin Response Factor ARF7. The interconnected activity of FLP and ARF7 thus could represent an important mechanism to stabilise the cellular auxin transport capacity, which is needed to sustain developmental processes such as lateral root initiation.