**Tuesday, June 21, 2016**

**5:30–5:45 pm Opening Ceremonies and Silver Medal Presentations**

**Exploring ABA receptors for water use-efficient plants**

Erwin Grill[1]
[1]*Technische Universität München*

Water deficit induces reduction of transpiration. Plants experiencing water deficit are able to improve carbon for water exchange leading to higher water use efficiency (WUE). Whether increased WUE can be achieved without trade-offs in plant growth is debated. The signals mediating the WUE response under water deficit are not fully elucidated but involve the phytohormone abscisic acid (ABA). ABA is perceived by a family of related receptors which are known to mediate acclimation responses and to reduce transpiration. We found that enhanced stimulation of ABA signalling via distinct ABA receptors can result in Arabidopsis plants constitutively growing at high WUE. Water productivity was associated with maintenance of net carbon assimilation, thereby sustaining biomass formation. The study shows that ABA receptors can be explored to generate more plant biomass per water transpired.

**Strigolactone biosynthesis and action in rice and Arabidopsis**

Shinjiro Yamaguchi[1]
[1]*Tohoku University*

Until recently, little was known about the strigolactone (SL) biosynthetic pathway. Recently, biochemical analysis of genetically identified components revealed that carlactone, which has an SL-like carbon skeleton, is produced from all-*trans*-β-carotene by sequential reactions of DWARF27 (D27), D17 (carotenoid cleavage dioxygenase7 [CCD7]) and D10 (CCD8) *in vitro*. Using $^{13}$C-carlactone, we showed that $^{13}$C-labeled carlactone is converted to $^{13}$C-*ent*-2'-*epi*-5-deoxystrigol (4-deoxyorobanchol; 4DO), an endogenous SL in rice, *in planta*. Quantitative analysis of endogenous carlactone in Arabidopsis showed that it accumulates to an extremely high level in the *max*1 mutant, which is defective in a cytochrome P450 monooxygenase. This result suggests that carlactone is a direct substrate for MAX1. In fact, we found that carlactone is converted to a carboxylated metabolite, named carlactonoic acid (CLA), in a MAX1-dependent manner in Arabidopsis. Moreover, $^{13}$C-CLA was converted to $^{13}$C-4DO in rice. In Arabidopsis, CLA was converted to its methyl ester (MeCLA). We found that MeCLA, but not CLA, was able to interact with Arabidopsis D14 (AtD14) protein, an SL receptor, *in vitro*. These results suggest that MeCLA is biologically active in inhibiting shoot branching in Arabidopsis.

**There and back again**

Peter McCourt[1]
[1]*Cell & Systems Biology, University of Toronto*

Over the past 20 years, my program has focused on how the hormone abscisic acid (ABA) modulates plant growth particularly during germination and early seeding growth. Although a core ABA signaling pathway now exists we are continuing to study ABA signaling with the idea of turning the core into a network using the tools of systems biology. My group has also recently begun a program to molecularly understand a second plant hormone, strigolactones (SLs). SLs are important chemical cues for seed germination, particularly in the parasitic plant *Striga*. Because of our experience in dissecting hormonal roles in seed germination we feel well positioned to address similar questions in this recently identified hormone. This research will not only provide fundamental insights into plant hormone function but should also make it easier to identify key components involved in ABA and SL signaling in other plant species including crops.

**Wednesday, June 22, 2016**

**9:00-10:35 am Plenary I: Hormone Metabolism & Transport**

**Regulation of synthesis and transport of cytokinins for quantitative and qualitative tuning of actions for plant growth optimization**

Hitoshi Sakakibara[1]
[1]*RIKEN CSRS*

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. We have demonstrated that *IPTs*, *CYP735As*, and *LOGs*, which are key genes for *de novo* CK biosynthesis, are expressed in various parts during growth and development, and differentially regulate the synthesis of $N^6$-($\Delta^2$-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. This regulation is one of the qualitative controls of CK action involved in shoot growth regulation by root-borne signal. In addition, *ABCG14*, a member of ABC transporter family, has been recently identified as a key gene for root-to-shoot translocation of CKs via xylem. The biosynthesis and transport genes are regulated by nutritional cues for linking its status to growth regulation. Our studies show that external (nitrate) and internal (Gln) N-status, and C-status (sugar) independently regulate *de novo* CK synthesis in phloem, where N and C-nutritional information are integrated, to convert metabolic signal to growth regulation signal. Furthermore, our recent studies suggest importance of translocation of active form CK via xylem (i.e. tZ) for regulation of specific traits in shoot growth. We will outline our recent progress in CK study, and discuss the physiological significance of regulation of CK action to optimize growth and development at whole plant level.

**11:00-12:30 am Plenary II: Novel Signaling Molecules**

**Identification of novel peptide hormones in plants**

Yoshikatsu Matsubayashi[1]
[1]*Department of Biological Science, Graduate School of Science Nagoya University*

Cell-to-cell signaling mediated by secreted ligands and membrane-localized receptors is one of the critical mechanisms by which growth and development of multicellular organisms are cooperatively regulated. Because membrane-localized receptors act as master switches of complex intracellular signaling, identification of the ligand-receptor pair is one of the central issues of post-genome research. Following complete sequencing of the *Arabidopsis* genome, a number of genes encoding small secreted peptides have been identified. We are working to clarify the mechanisms by which plant development is regulated, through identification of novel ligands such as small secreted peptides and their specific receptors using *Arabidopsis* genome information, biochemical analysis and phenotypic observation.

Root meristem growth factor (RGF) is a 13-amino-acid peptide that regulates root meristem development through the PLETHORA (PLT) stem cell transcription factor pathway. RGF family peptides are expressed in stem cell area in the root tip and create a diffusion-based concentration gradient in the root meristem. RGF is recognized by an LRR-receptor kinase (LRR-RK), RGFR, and defines PLT expression in the proximal meristem, thereby acting as a key regulator of root meristem patterning.

C-terminally encoded peptide (CEP) is a 15-amino-acid peptide that mediates long-distance nitrogen (N)-demand signaling. When external N availability is lowered, CEP expression is promptly upregulated in roots. CEP acts as a root-derived ascending N-demand signal to the shoot, where its perception by an LRR-RK, CEPR, leads

to the production of a putative shoot-derived descending signal that upregulates nitrate transporter genes in the distant part of the roots. This mechanism supports N acquisition when nitrate is unevenly distributed within the soil.

**References**

(1) Tabata R., Sumida K., Yoshii T., Ohyama K., Shinohara H., Matsubayashi Y. Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling ***Science*** 346, 343-346 (2014)

(2) Matsuzaki Y., Ogawa-Ohnishi M., Mori A., Matsubayashi Y. Secreted peptide signals required for maintenance of root stem cell niche in *Arabidopsis*.
***Science*** 329, 1065-1067 (2010)

**Antheridiogen determines sex in ferns via a spatiotemporally split gibberellin synthesis pathway**

Junmu Tanaka[1], Makoto Matsuoka[1] and Miyako Ueguchi-Tanaka[1]
[1]*Bioscience and Biotechnology Center, Nagoya University*

Some ferns possess the ability to control their sex ratio in their colony with the aid of antheridiogen. Antheridiogens are antheridium (male organ)-inducing compounds with structural similarity to gibberellin. We determined that ferns have evolved an antheridiogen-mediated communication system to produce males by modifying the gibberellin biosynthetic pathway, which is split between two individuals of different developmental stages in the colony. Antheridiogen acts as a bridge between them because it is more readily taken up by prothalli than bioactive gibberellin, which may due to its hydrophobic properties. The pathway initiates in early-maturing prothalli (gametophytes) within a colony, which produce antheridiogens and secrete them into the environment. After the secreted antheridiogen is absorbed by neighboring late-maturing prothalli, it is modified into bioactive gibberellin to trigger male organ formation.

**Binding of RALF1 to the FERONIA receptor kinase downregulates the plasma membrane $H^+$- ATPase and reduces cell elongation in roots**

Miyoshi Haruta[1], Vilas Gaddameedi[1], Grzegorz Sabat[1], Donna Fernandez[1], and Michael R. Sussman[1]
[1]*Department of Biochemistry, Biotechnology Center, Department of Botany, University of Wisconsin-Madison*

Cell elongation is an essential process for plant growth and development. A 5-kDa peptide, Rapid Alkalinization Factor 1 (RALF1) regulates root cell elongation via binding to a receptor kinase, FERONIA. Using an *in vitro* fluorescent polarization binding assay we have now demonstrated that RALF binding to the FERONIA ectodomain is sequence specific, reversible, dose responsive, and pH dependent. The effect of a kinase negative mutation of FERONIA on root growth was compared to the effects of this mutation on fertilization. While the kinase negative mutation does not affect fertilization, reduced kinase activity results in reduced sensitivity to RALF1-induced root growth inhibition, indicating that there may be differences in the signaling pathway utilized in reproductive versus vegetative tissue. The molecular mechanism for changes in RALF1 sensitivity of the kinase-negative mutant is also being interrogated by measuring changes in RALF1-induced cytoplasmic calcium concentration. RALF1-induced phosphorylation changes in a plasma membrane proton pump, AHA2, correlates with the peptide's ability to induce extracellular alkalinization. To genetically test AHA2 involvement in RALF-FERONIA physiology, we examined the growth of plants containing mutations in AHA2. A phosphomimetic mutation of AHA2 at Ser899, an amino acid whose phosphorylation was increased by RALF, caused reduced ability to rescue the *aha2* mutant. Moreover, a double mutation, *fer/aha2*, suppresses the longer root phenotype seen with *feronia* single mutants. AHA2 acting downstream of RALF-FERONIA pathway is also supported by an observation that AHA2 protein abundance decreases in WT when treated with RALF1. This AHA2 abundance change was not seen in *feronia* mutant. Overall, our results support a model in which FERONIA and RALF1 kinase signaling restricts cell expansion by down-regulating AHA2 function.

**2:00-3:30 pm Concurrent 1A: Hormone Interaction**

**The pea branching *RMS2* gene encodes the PsAFB4/5 auxin receptor and is involved in an auxin-strigolactone regulation loop**

Yasmine Ligerot[1], Alexandre De Saint Germain[1, 7], Sylvie Citerne[1], N. Kadakia [3], Romain Novaretti[1], Jean-Paul Pillot[1], Cristelle Troadec[4], Tanya Waldie[2], Grégoire Aubert[6], Frédéric Debellé[5], Abdel Bendahmane[4], Mark Estelle[3], Ottoline Leyser[2] and Catherine Rameau[1]

*[1]Institut Jean-Pierre Bourgin, [2]Sainsbury Laboratory, University of Cambridge, [3]Section of Cell and Developmental Biology and Howard Hughes Medical Institute, University of California San Diego, [4]Institut des Sciences des Plantes de Paris, [5]Laboratoire des Interactions Plantes–Microorganismes, INRA/CNRS, [6]INRA, [7]Plant Biology Laboratory, The Salk Institute for Biological Studies*

Control of shoot branching involves auxin, cytokinin and strigolactones (SLs). In pea, most of the high branching *ramosus* (*rms*) mutants show high expression of the SL biosynthesis *RMS1/CCD8* gene and low xylem-sap cytokinin content. In contrast, the high branching *rms2* mutant displays very low expression of *RMS1* and high xylem-sap cytokinin content. Previous physiological characterization of the pea *rms2* mutant suggested that *rms2* was affected in a shoot-to-root feedback signal controlling both SL biosynthesis and cytokinin level in the xylem sap.

The nature of the feedback signal was investigated by cloning the pea *RMS2* gene. We showed that *RMS2 encodes* the pea ortholog of the Arabidopsis auxin receptor belonging to the AUXIN-SIGNALING F-BOX4 (AFB4)/AFB5 clade suggesting that the shoot-to-root feedback signal is very likely auxin. To investigate whether the pea RMS2 and AFB4/5 from Arabidopsis have the same functions, we analyzed *rms2* phenotypes (e.g. shoot branching, high IAA levels) in the Arabidopsis *afb4, afb5* and *afb4 afb5* double mutants and reciprocally, we tested whether the pea *rms2* mutants showed the high selective resistance to the herbicidal auxin picloram of the *afb5* mutant. Complementation studies and biochemical analysis were also performed.

The *rms* branching mutants, rather than being depleted in IAA levels, contained elevated IAA levels. We proposed a model where a non-response to SLs, which occurs in SL-biosynthesis or in SL-response *rms* mutants, stimulates the synthesis of a feedback signal, auxin, which controls via RMS2, CK levels in the xylem sap and SL biosynthesis gene expression. We tested this model by quantifying IAA levels after SL treatment using the pea SL *rms* mutants. We demonstrated that SLs repress IAA levels by down-regulating transcript levels of auxin biosynthesis genes in stem (*TAR2* and *YUC1*) independently of polar auxin transport.

**Interaction of cytokinin with auxin and ethylene in the control of primary root growth**

Ian Street[1], Dennis Mathews[2], Sitwat Aman[1], Maria Yamburenko[1], Yan Zubo[1], Ranjan Swarup[3], Malcolm Bennett[3], Samina Shakeel[1], Joseph Kieber[4], and G. Eric Schaller[1]

*[1]Dartmouth College, [2]University of New Hampshire, [3]University of Nottingham, [4]University of North Carolina-Chapel Hill*

Cytokinin inhibits primary root growth in Arabidopsis through effects on both cell elongation and cell proliferation. Inhibition of cell elongation by cytokinin requires the auxin importer AUX1, *AUX1* mutants specifically affecting the ability of cytokinin to inhibit cell elongation but not cell proliferation. AUX1 is required for cytokinin-dependent changes of auxin activity in the lateral root cap and epidermal layer of the transition zone. Cytokinin directly regulates expression of *AUX1*, pointing to a mechanism by which cytokinin can alter auxin transport and auxin activity. The regulation of root cell elongation by cytokinin operates through ethylene-dependent and independent mechanisms, both hormonal signals converging on AUX1 as a regulatory hub. Inhibition of root cell proliferation by cytokinin was previously shown to involve SHY2, a negative regulator of auxin signaling. Recently ethylene has been determined to regulate cell proliferation as well as cell elongation at the root tip and, like cytokinin, converges on the regulation SHY2 as a mechanism to reduce auxin signaling and inhibit cell proliferation. Mutant-based analysis indicates that ethylene contributes to the effects of cytokinin in

the inhibition of cell proliferation. Our results support a general model for the control of primary root growth that involves two main features: (1) independent roles for shootward auxin transport in the control of cell elongation and of rootward auxin transport in the control of cell proliferation; and (2) convergence of the phytohormones cytokinin and ethylene on a shared set of targets to regulate auxin activity and thus cell proliferation and elongation in the primary root.

**Regulation of the growth hormone networks by the endogenous circadian clock and sugar signals**

Zhi-Yong Wang[1], Eunkyoo Oh[1], Jia-Ying Zhu[1], and Zhenzhen Zhang[1]
*[1]Department of Plant Biology, Carnegie Institution for Science, [2]College of Life Science, Hebei Normal University, Shijiazhuang*

While proper growth responses are required to optimize light exposure and photosynthetic efficiency, plant growth also requires photosynthates as energy source and call wall building materials. Therefore, hormone programs need to respond to not only environmental signals such as light and temperature, but also endogenous cues such as nutrient availability and circadian rhythms. Shoot cell elongation is synergistically promoted by growth hormones brassinosteroid (BR), auxin, and gibberellin (GA), and inhibited by light signals, through cooperative interactions among the BR-activated BZR1 family transcription factors, the auxin response factors (ARF), and the phytochrome-interacting factors (PIFs), as well as their antagonism by the GA-sensitive DELLA proteins (BAP/D module). The BAP/D module elegantly explains how these hormonal and light signals co-regulate shoot cell elongation and seedling photomorphogenesis, and how additional cues such as the circadian clock and temperature control hormone sensitivities by altering PIF levels. Here we report that (1) the circadian clock gate plant growth through direct interaction between the clock component TOC1 and the PIF4 transcription factor, to provide optimal thermo-responsive growth at the time of natural heat stresses, (2) sugar signalling through the Target Of Rapamycin (TOR) pathway controls the accumulation of BZR1 to balance growth with photosynthate availability. Our studies further expand our view of the hormone interaction network that control plant growth.

**2:00-3:30 pm Concurrent 1B: Abiotic Interactions**

**Ethylene-gibberellin relay induces internode elongation in deepwater rice**

Motoyuki Ashikari[1]
*[1]Nagoya University*

In general, the rice plant is adapted to shallow flooding but it cannot survive deepwater condition because of anoxia. In Asia and West Africa, floodingdue to heavy rains is common during the rainy season. Although flooding is an adverse environmental condition for rice, deepwater rice can survive under prolonged flooding via drastic internode elongation. The leaves that remain above the water surface allow the proper respiration of the plant. QTL analysis has been carried out to elucidate the mechanism of internode elongation in deepwater rice under prolonged submergence. The results show that three major QTLs regulate internode elongation, one of which encodes a gibberellin (GA) biosynthesis gene for internode elongation under deepwater condition. The gene is strongly induced in deepwater rice during prolonged submergence, compared to non-deepwater rice (normal rice) subjected to a similar condition. Deepwater rice accumulates ethylene under prolonged submergence. Ethylene application also induces the GA biosynthesis gene under normal (air) condition. We found that binding of transcription factors for ethylene signaling directly activates the promoter of this gene. Furthermore, the promoter region of this gene is conserved in deepwater rice lines showing strong internode elongation in response to submergence. These results suggest that an ethylene-regulated induction system mechanism of the GA biosynthesis gene contributes to internode elongation in submerged deepwater rice.

**Release of GTP exchange factor mediated regulation of abscisic acid signal transduction through ABA-induced rapid processing of RopGEFs**

Zixing Li[1], Rainer Waadt[1], and Julian I. Schroeder[1]
[1]*Division of Biological Sciences, Cell and Developmental Biology Section, University of California San Diego*

Abiotic stress leads to activation of ABA signal transduction, which is mediated by the core components: PYL/RCAR ABA receptors, PP2C-type protein phosphatases and protein kinases. Small GTPases of the ROP/RAC family act as negative regulators of ABA signal transduction. However, the mechanisms by which ABA controls the behavior of ROP/RACs have remained unclear. We will present data showing that an *Arabidopsis* guanine nucleotide exchange factor RopGEF protein is rapidly sequestered to intracellular particles in response to ABA and is degraded. Interestingly, RopGEF1 directly interacts with specific PP2C protein phosphatases and undergoes constitutive processing in higher order *pp2c* mutant plants, revealing that active PP2C protein phosphatases protect and stabilize RopGEF1 from translocation. In addition we have found that ABA-mediated processing of RopGEF1 also plays an important role in ABA-mediated regulation of lateral root growth. The presented findings point to a PP2C-RopGEF-ROP/RAC control loop model that is proposed to aid in shutting off ABA signal transduction, to counteract leaky ABA signal transduction caused by "monomeric" PYL/RCAR ABA receptors in the absence of stress and amplifies signaling in response to ABA.

**Novel Epigenetic, RNA and Peptide Regulation in PlantAbiotic Stress Responses**

Jong-Myong Kim[1], Akihiro Matsui[1], Kentaro Nakaminami[1], Kaori Sako[1,2], Minoru Ueda[1,2], Taiko Kim To[1,3], Huong Mai Nguyen[1,4], Khurram Bashir[1,2], Sultana Rasheed[1,4], Junko Ishida[1], Maho Tanaka[1], Minoru Yoshida[1], Masanori Okamoto[1,5], Kousuke Hanada[6], Sachihiro Matsunaga[2,7], Yoshiki Habu[2,8] and Motoaki Seki[1,2,4]
[1]*RIKEN CSRS,* [2]*CREST, JST,* [3]*University of Tokyo,* [4]*Yokohama City University,* [5]*Tottori University,* [6]*Kyushu Institute of Technology,* [7]*Tokyo University of Science,* [8]*NIAS,*

Plants respond and adapt to drought, heat, cold and high-salinity stresses in order to survive. Many abiotic stress-regulated genes have been identified by genetic and transcriptome analysis, and its function in the stress responses has been elucidated. However, we think that novel mechanisms involving epigenetic, RNA and peptide regulation have additional functions.
Recently, we found that the following novel regulation mechanisms function in plant abiotic stress responses. 1) *Arabidopsis* Histone Deacetylase 6 (HDA6) is a master regulator of novel drought stress response network. HDA6 regulates conversion of central metabolic pathway from glycolysis to acetic acid biosynthesis under water deficit condition. Furthermore, acetic acid pretreatment enhances plant drought tolerance. 2) Treatment with histone deacetylase (HDAC) inhibitors enhances high-salinity stress tolerance. Ky-2, a HDAC inhibitor, enhances the salinity stress tolerance via regulation of SOS1 in *Arabidopsis.* 3) AT13 peptide functions in high-salinity stress tolerance. 4) Abiotic stress-responsive non- coding antisense RNAs are synthesized from sense transcripts of protein-coding genes without the involvement of siRNA biosynthesis by RNA-dependent RNA polymerases (RDRs) and function in drought stress adaptation.
In this meeting, I will present our recent findings in the abiotic stress adaptation.

**Tissue-specific regulation of gibberellin signaling fine-tunes the iron availability responses**

Michael Wild[1], Jean-Michel Davière[2], Grégory Vert[2] and Patrick Achard[2]
*[1]University of Freiburg, [2]Institut de Biologie Moléculaire des Plantes*

Iron deficiency is one of the most common micronutrient deficiencies in the world, and World Health Organization estimates that more than 10% of the world population suffers from iron malnutrition. Plant foods are the principal iron source for humans. Hence, improving iron content and bioavailability in plant food products would be an efficient and economical way to fight human iron malnutrition.
Plants have evolved efficient mechanisms to cope with iron-deficiency and various phytohormones have been implicated (Kobayashi and Nishizawa, 2012). Gibberellins (GAs) are a class of plant growth-promoting hormones that play important roles throughout plant development, including seed germination, growth, floral transition, and in many aspects of the adaptation of plant growth in response to environmental variable inputs. GAs control a wide range of processes by opposing the function of the DELLA proteins, a family of nuclear growth repressors (Peng et al., 1997). When GA levels are low, DELLAs accumulate and modulate the activity of key regulatory proteins, including members of the bHLH family of transcription factors (TFs), involved in diverse pathways. (Davière and Achard, 2013).
Our data show that the GA-signaling DELLA repressors contribute substantially in the adaptive responses to iron-deficient conditions. In *Arabidopsis*, *FER-like IRON-DEFICIENCY INDUCED TRANSCRIPTION FACTOR* (*FIT*) encodes a bHLH-TF that activates the expression of iron uptake machinery genes in root epidermis upon iron deficiency (Colangelo and Guerinot, 2004). We demonstrate that DELLAs physically interact with FIT to prevent its binding to target promoters, inhibiting its transcriptional activity. Limited iron availability antagonizes such repression by reducing DELLA abundance in epidermal cells in the root differentiation zone and as a consequence fully activates FIT downstream target gene expression and thus iron uptake. Overall, our data highlight that spatial distribution of DELLAs in roots is essential to fine-tune the adaptive responses to iron availability.

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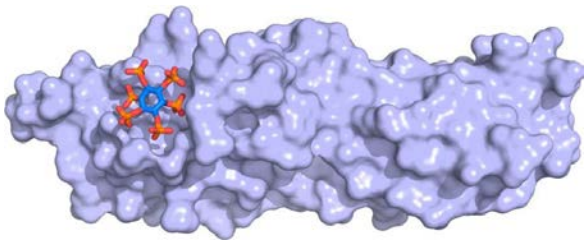
**Control of plant phosphate homeostasis by SPX inositol polyphosphate sensor domains**

Rebekka Wild[1], Ruta Gerasimaite[2], Ji-Yul Jung[3], Vincent Truffault[4], Igor Pavlovic[5], Henning Jacob Jessen[5,6], Yves Poirier[3], Andreas Mayer[2], and Michael Hothorn[1]
*[1]Structural Plant Biology Laboratory, Department of Botany and Plant Biology, University of Geneva, [2]Department of Biochemistry, University of Lausanne, [3]Department of Plant Molecular Biology, University of Lausanne, [4]Department of Biochemistry, Max Planck Institute for Developmental Biology, Tübingen, [5]Department of Chemistry and Pharmacy, University of Zürich, [6]Institute of Organic Chemistry, Albert-Ludwigs-University Freiburg, Germany.*

Phosphate is an important macronutrient and thus eukaryotic cells tightly regulate their intracellular phosphate ($P_i$) levels. $P_i$ homeostasis can be maintained by adapting phosphate uptake, storage and transport. However, it is poorly understood how cells sense and signal their phosphate status.
SPX domains are found at the N-terminus of eukaryotic phosphate transporters, inorganic polyphosphate polymerases and signaling proteins. I will present structural, biochemical and genetic evidence that SPX domains

are sensors for inositol polyphosphate (InsP) ligands, signaling molecules whose concentration change in response to phosphate availability. Mutations in the SPX InsP binding pocket impair InsP binding in biochemical binding assays, down-regulate synthesis of inorganic polyphosphate in yeast and reduce phosphate transport in Arabidopsis. Further, InPs trigger the interaction between stand-alone plant SPX proteins with a family of phosphate-starvation responsive transcription factors, thereby controlling the induction of phosphate starvation responses under low $P_i$. Taking together, we suggest that InsPs act as novel signaling molecules in fungi, plants and animals.
Binding of InsPs allows SPX domains to bind and regulate their downstream signaling partners and thus to regulate phosphate homeostasis in eukaryotes.



3D structure of the InsP ligand bound to the SPX sensor domain

**Enhancement of ABA receptor confers water-saving drought tolerance in wheat**

Masanori Okamoto[1,2], Fumitaka Abe[3], June-Sik Kim[1], Keisuke Tanaka[4], Hisato Kobayashi[4], Yoichi Sakata[5], Kousuke Hanada[6] and Ryosuke Mega[1]
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Drought stress causes to reduce crop production, and available water for agriculture is restricted in arid region. Therefore, drought tolerant and water-saving crops are required for stable food supply. Abscisic acid (ABA) contributes to drought tolerance in plants. Soluble PYR/PYL receptors, which belong to the START family protein, exist across higher plants. Thus, it is thought that PYR/PYL receptors can be a target gene to improve drought tolerance of plant. We generated transgenic wheat overexpressing wheat PYR/PYL (TaPYLox) and investigated drought tolerance and the other physiological traits. Seedlings of TaPYLox showed ABA hypersensitive phenotype. Transcriptome analysis revealed that many ABA responsive genes were induced in TaPYLox even under the well-watered condition, indicating that TaPYLox already possesses drought tolerant traits prior to drought stress. In addition, stomatal conductance and transpiration rate were decreased in TaPYLox. Water-use efficiency of TaPYLox calculated from the rate of photosynthesis and transpiration was improved compared with control plant. Moreover, biomass amount and seed yield produced from 1L of water significantly were increased in TaPYLox. Therefore, enhancement of ABA receptor expression contributes to not only drought tolerance but the “water-saving drought tolerance” phenotype, which can perform highly efficient $CO_2$ fixation under the limited water condition.

**2:00-3:30 pm Concurrent 1C: Hormone Perception**

**Strigolactone perception by DAD2 and the environmental control ofbranching**

**Kim C Snowden[1], Revel SM Drummond[1], Cyril Hamiaux[1], Zhiwei Luo[1], Hui Wen Lee, and Bart J Janssen[1]**
[1]*The New Zealand Institute for Plant & Food Research*

The strigolactone hormone signalling system of plants controls the number of branches produced and responds to nutrient status and light. Data will be presented that are consistent with DAD2 being the strigolactone receptor. Unusually for a hormone receptor, DAD also has enzyme activity and can hydolyse its ligand. We have undertaken mutagenesis of DAD2 to dissect the relative contributions of enzyme activity and interactions with signal transduction partners to understand how strigolactones are perceived. We have also exploited the features of the DAD2 protein to undertake screens for antagonists of SL perception. In addition, DAD2 is postulated to act at a position in the network controlling axillary bud outgrowth that integrates nutrient availability and light quality. We investigated the relative importance of these two factors by simultaneously altering both light and nutrient conditions (red:far-red ratio and phosphate availability). An analysis of gene expression of SL pathway genes showed a co-ordinated response to phosphate and light and that the regulation of the SL receptor plays an important role in the response of plants to the environment.

**A mechanism of rapid ABA signaling inactivation through tyrosine nitration of PYR/PYL/RCAR receptors**

José León[1]
[1]*Instituto de Biología Molecular y Celular de Plantas (Consejo Superior de Investigaciones Científicas – Universidad Politécnica de Valencia)*

ABA induces nitric oxide (NO) production in plants, and NO has been considered to be a required intermediate in ABA triggered responses such as stomata closure. However, we found that the triple *nia1nia2noa1-2* mutant plants, with low endogenous NO content, were hypersensitive to ABA in seed germination, stomata closure or tolerance to water deficit, thus suggesting NO acts somehow as a negative regulator of ABA signaling. We identified a mechanism by which NO exerts a rapid and negative regulation on ABA signaling that is based on the nitration of key Tyr residues of PYR/PYL/RCAR receptors. *In vitro* nitration receptors led to their inability in inhibiting phosphatase activity thus suggesting nitration of Tyr residues made receptors inactivated. Inactivation was specifically due to Tyr nitration because the same receptors can be also S-nitrosylated *in vitro* but S-nitrosylated forms were fully active in binding ABA and further inhibiting phosphatase activity. The use of recombinant proteins and *in vitro* induced NO-related PTMs was further complemented by an *in planta* protemic approach based on the use of transgenic plants expressing tagged versions of PYR/PYL/RCAR receptors, immunopurification procedures based on anti- tag-coated magnetic beads and LC-MS/MS techniques. We found that PYR/PYL/RCAR receptors are nitrated in Tyr, S-nitrosylated in Cys and also ubiquitylated in Lys residues *in vivo.* We also identified the modification sites for PYR1, PYL4 and PYL8 receptors *in planta*. Nitration of Tyr residues and polyubiquitylation of the nitrated population of receptor molecules seems to be linked, and prepare the modified protein for further proteasomal degradation, which is on the basis of the mechanism allowing the desensitation to ABA and the subsequent resetting. This mechanism of rapid inactivation of the ABA receptors through Tyr nitration events could be especially useful for those conditions requiring faster responses than those based on the catabolism of the hormone.

**Probing strigolactone receptors in Striga hermonthica with fluorescence**

Yuichiro Tsuchiya[1]
[1]*Nagoya University*

Damages caused by the parasitic plant Striga hermonthica comprise the largest impediment to securing food for sustaining the explosively growing population in Africa, which leads to the loss of 10 billion US dollars worth of crops from the continent every year1. Since the discovery of strigol in the 1960's as a host-derived germination stimulant for Striga, elucidating the mechanism responsible for perceiving the group of related molecules, strigolactones (SLs), has been the central point of interest to control the Striga problem. Nevertheless, the molecular identity of the SL receptor in Striga remains unknown, as this obligate parasite is intractable by conventional genetic studies. Here, we describe a chemical genetic approach to investigate SL receptors using a fluorescence turn-on probe, Yoshimulactone Green (YLG), that enables rapid identification, characterization and visualization of SL receptors in Striga. Using YLG, we identified highly diverged α/β hydrolase-fold proteins including functional SL receptors in Striga. Moreover, the fluorescence turn-on functionality in YLG enabled tracking of the signal perception by SL receptors in vivo. Live-imaging experiments revealed a wave-like propagation of perception that wakes up Striga seeds. Overall, these results open an avenue to access SL receptors and regulatory dynamics of SL signal transduction in Striga, which will potentially provide a powerful solution to the Striga problem.

**Understanding Auxin perception and selectivity**

Mussa Quareshy1 and Richard Napier[1]
[1]*University of Warwick*

Auxin (Indole-3-acetic acid) can be considered one of the most important hormones in plant development as it coordinates plant responses through transcriptional regulation. Auxin binds Transport Inhibitor Response 1 (TIR1) of which there are 5 other homologues; Auxin Signalling F-Box (AFB1 – 5). TIR1 and AFB5 are the most distantly related in terms of sequence homology and are studied in this work.
Currently 25 molecules are marketed as synthetic auxins and there is still a drive to discover new auxin-like molecules, in particular from an agrochemical perspective to overcome weed-based resistance and reduce field dose. Interestingly, there is as yet no definitive descriptor that defines an auxin chemically and until recently there has been no compound that has complete selectivity for one of the individual receptor proteins.
We will present our work using purified TIR1 an AFB5 and compound screening by SPR, *in-silico* docking, kinetic parameterization and 3D QSAR modelling to identify and synthesize potential novel auxins or anti-auxins. This has yielded a novel class of auxin molecule, which has also shown selectivity between some of the auxin receptors. It is a potential scaffold for receptor-specific auxins and thus a new generation of herbicides. We also present our work on auxin- molecular field descriptors as a tool in the search for rationally designed novel auxins.

**The D14 strigolactone receptor: part-enzyme part-receptor**

Alexandre de Saint Germain[1], Catherine Rameau[2] and François-Didier Boyer[2,3], and Joanne Chory[1]
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Strigolactones (SLs) are a class of plant hormones that control plant architecture and participate in parasitic and symbiotic interactions in the rhizosphere[1]. Recent studies on strigolactone (SL) perception and signaling have identified the putative receptor D14, the D14-interacting F-box protein MAX2, and possible targets of MAX2-mediated degradation including D53[2]. SL receptors (AtD14 in *Arabidopsis*) belong to the α/β-fold hydrolase

superfamily and contain the Serine, Histidine, Aspartate catalytic triad located in an hydrophobic active site pocket. X-ray structures and enzymatic assays revealed that GR24 (a synthetic SL analogue) is hydrolyzed by a nucleophilic attack by the Serine residue leading to two inactive products[3,4]. Despite that, the role of SL degradation and D14 catalytic activity in the signaling process is not been well understood.
In our recent work[5], we combined genetic, physiological and biochemical approaches to uncover the mechanism and the function of SL degradation by D14. We generated bioactive profluorescent probes to monitor enzyme kinetics which allowed us to demonstrate that the receptor acts as a single turnover enzyme. We propose a model where the hydrophobic ABC part of the SL facilitates the positioning of the D ring within the catalytic triad, the SL is hydrolyzed, and a covalent receptor/D-ring complex is formed which initiates signaling by destabilization and/or surface change of the receptor.


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3 Hamiaux, C. *et al.* DAD2 Is an alpha/beta Hydrolase Likely to Be Involved in the Perception of the Plant Branching Hormone, Strigolactone. *Curr. Biol.* 22, 2032-2036, (2012).
4 Zhao, L. H. *et al.* Crystal structures of two phytohormone signal-transducing alpha/beta hydrolases: karrikin- signaling KAI2 and strigolactone-signaling DWARF14. *Cell Res* 23, 436-439, (2013).
5 **de Saint Germain, A**. *et al.* An histidine covalent receptor/butenolide complex is involved in strigolactone perception. Nature chemical biology, accepted, (2016).


**Ligand-induced transitions in the phosphorylation status of ethylene receptors in tomato fruit.**


Yusuke Kamiyoshihara[1], Denise M. Tieman[2], Donald J. Huber[2], and Harry J. Klee[2]
*[1]College of Bioresource Science, Nihon University, [2]Horticultural Sciences Department, University of Florida*


The plant hormone ethylene is perceived by a membrane-associated receptor family which is similar to the bacterial two-component histidine kinase receptors. Since ethylene receptors negatively regulate the signaling, the suppression is canceled upon ethylene binding, permitting responses including fruit ripening. Although receptors have autophosphorylation activity, the mechanism whereby signal transduction occurs has not been fully understood. Here we demonstrate that SlETR4, an important receptor for tomato (*Solanum lycopersicum*) fruit ripening, is multiply phosphorylated in vivo and the phosphorylation level is dependent on ripening stage and ethylene action. Although only phosphorylated isotypes were detected in immature and mature green fruits, the non-phosphorylated isotype appeared after ripening started. Furthermore, treatment of preclimacteric fruits with ethylene resulted in accumulation of SlETR4 with reduced phosphorylation while treatments of ripening fruits with ethylene antagonists, 1-methylcyclopropene and 2,5- norbornadiene, induced accumulation of the phosphorylated isotypes. A similar phosphorylation pattern was also found for Never ripe (Nr=SlETR3), another ripening-related receptor. Alteration in the phosphorylation state of receptors is likely to be an initial response upon ethylene binding since treatments with ethylene and 1-methylcyclopropene rapidly influenced the phosphorylation state. The SlETR4 phosphorylation state was closely related to ripening progress, suggesting that the phosphorylation state of receptors is implicated in the ethylene signal output in the fruits. This phosphorylation may act as a regulator for the interaction with downstream components of signal transduction.

**4:00-5:30 pm Concurrent 2A: Hormone Metabolism**

**Auxin biosynthesis in Arabidopsis**

Yunde Zhao[1]
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It has been proposed that auxin is synthesized from both Trp-dependent and Trp-independent pathways. Whereas the understanding of Trp-independent pathway is still very limited, much progress has been made in Trp-dependent pathway. It is generally recognized that the TAA/YUC pathway, in which Trp is first converted into Indole-3-pyruvate (IPA) by the TAA family of aminotransferases and IPA is subsequently converted to IAA by the YUC family of monooxygenases, is a main auxin biosynthetic pathway and plays essential roles in all major developmental processes. Here I will discuss our recent progress in regulation of the TAA/YUC pathway. I will also discuss whether there exist other Trp-dependent pathways and whether the other proposed Trp-dependent pathways play important roles in plant growth and development. Furthermore, I will report the new tools we have developed in manipulating auxin biosynthesis in Arabidopsis.

**Regulation of gibberellin catabolism by touch**

Theo Lange[1] and Maria João Pimenta Lange[1]
[1]*Braunschweig University of Technology, Institute of Plant Biology*

Touch-induced morphological changes in plants, called thigmomorphogenesis, include stunted growth and delay in flowering. In Arabidopsis, these changes have been associated to the phytohormone jasmonate (JA). However, those phenotypes are reminiscent of plants deficient in the phytohormone gibberellin (GA). Recently, we show that touch-induced morphological changes in Arabidopsis are GA-regulated and JA-independent. Endogenous GA-levels were analysed by gas chromatography-mass spectrometry, and transcript levels of GA-, JA-, and touch related genes were quantified by reverse-transcriptase polymerase chain reaction. Touch reduces endogenous GA-levels and, moreover, the resulting morphological changes can be restored by exogenous application of bioactive GA4. Furthermore touch induces expression of the gene AtGA2ox7, encoding an enzyme involved in GA-catabolism, and Arabidopsis ga2ox7 loss-of-function mutants do not respond to touch, identifying this gene as a key-regulator for thigmomorphogenesis. Re-characterisation of recombinant AtGA2ox7 revealed new properties for this enzyme that will be discussed.

**SOL1 and other peptidases are responsible for CLE peptide processing mechanisms**

Reira Suzuki[1], Morihiro Oota[1], Chie Shimaoka[1], and Shinichiro Sawa[1]
[1]*Kumamoto University*

Plant growth is strictly regulated by numerous signaling systems. Intercellular communication and subsequent intracellular signaling are known to be involved in the regulation of plant growth. In recent decades, small, secreted peptide hormones have been found to play essential roles in these intercellular signaling pathways together with phytohormones.
Plant peptide genes encode small secretory proteins, and are widely distributed in higher plants. Most peptide hormones contain signal peptide sequences that are cleaved during exocytosis-based secretion and transportation outside the cell. Moreover, the active domain of the mature peptide hormone is excised by proteolytic processing. Some peptide hormones undergo further posttranslational modification.
SUPPRESSOR OF LLP1 1 (SOL1), a putative $Zn^{2+}$ carboxypeptidase, has been found to exhibit proteolytic activity toward CLE peptides. SOL1 removes a C-terminal arginine residue from the CLE19 pro-peptide, and the removal of this residue confers full activity on the peptide. Here we will talk about CLE peptide processing mechanisms and its functions.

**Oxidative inactivation of auxin by DAO1 regulates growth in *Arabidopsis thaliana***

Jun Zhang[1], Jinshan Lin[2], Chinchu Harris[1], Fernanda Campos Mastrotti Pereira[3], Fan Wu[1], Joshua Blakeslee[2] and Wendy Peer[1]

*[1]University of Maryland, [2]The Ohio State University, [3]São Paulo State University*

Tight homeostatic regulation of the phytohormone auxin (indole-3-acetic acid, IAA) is essential to plant growth and survival. The *Arabidopsis thaliana* enzymes that function in auxin biosynthesis and conjugation to sugars and amino acids for temporary or permanent inactivation have been identified, but the enzyme that catalyzes oxidation of IAA to its primary catabolite 2- oxindole-3-acetic acid (oxIAA) remains uncharacterized. Here we show that DIOXYGENASE for AUXIN OXIDATION1 (DAO1) catalyzes formation of oxIAA *in vitro* and *in vivo* and that this mechanism regulates auxin homeostasis and plant growth. Null *dao1-1* mutants contain 95% less oxIAA compared to wild type, and complementation of *dao1* restores wild-type oxIAA levels, indicating that DAO1 is the primary IAA oxidase in seedlings. Further, *dao1-1* plants have phenotypes associated with increased auxin levels including elongated organs (hypocotyls, primary roots, rosette leaves, inflorescence stems), increased lateral root density and delayed sepal opening compared to wild type. These phenotypes are complemented by transformation with *DAO1pro*:YFP-DAO1. The dominant *dao1-2D* overexpression line has increased oxIAA levels, thus supporting DAO1 IAA oxidase function *in vivo*. *DAO1pro*:YFP-DAO1 expressed in *dao1-1* produces signals in the root tip as well as in all juvenile and mature vascular and epidermal tissues, especially in the sepal. A second isoform, DAO2, is very weakly expressed in seedling root apices. Together, these data confirm that IAA oxidation by DAO1 is the principal auxin catabolic process in Arabidopsis and that DAO1 is an important regulator of auxin homeostasis during plant morphogenesis.

**BIOCHEMICAL CHARACTERIZATION OF MORE AXILLARY GROWTH1 IN STRIGOLACTONE BIOSYNTHESIS**

Takahito Nomura[1], Kaori Yoneyama[1], Kohki Akiyama[2], Xiaonan Xie[1], Toshiyuki Ohnishi[3], Shinjiro Yamaguchi[4], and Koichi Yoneyama[1]

*[1]Utsunomiya University, [2]Osaka Prefecture University, [3]Shizuoka University, [4]Tohoku University*

Strigolactones (SLs) function as host recognition signals for root parasitic plants and symbiotic arbuscular mycorrhizal fungi in the rhizosphere and as plant hormones regulating shoot and root architecture inplants. CarotenoidisomeraseD27,carotenoidcleavagedioxygenasesCCD7 and CCD8, and cytochrome P450 monooxygenase MORE AXILLARY GROWTH1 (MAX1) were identified as SL biosynthesis enzymes by genetic screening of shoot branching mutants. D27, CCD7 and CCD8 convert β-carotene to carlactone (CL), an SL precursor having no canonical four-ring structure of SLs, by their sequential reactions. We have reported that *Arabidopsis* MAX1 catalyzes oxidation of the C-19 methyl group of CL to carboxylic acid, affording carlactonoic acid (CLA), while one of rice MAX1 homologs was reported to catalyze the conversion of CL to 4-deoxyorobanchol having the four-ring structure. In order to know which is the common reaction in MAX1 homologs is, we examined the enzymatic functions of MAX1 homologs in Arabidopsis, rice, maize and tomato using a yeast expression system. As a result, the conversion of CL to CLA was found to be a common function in MAX1 homologs but not that of CL to 4DO.

**Jasmonoyl-isoleucine catabolic pathways provide new insights into jasmonate homeostasis**

Thierry Heitz[1], Ekaterina Smirnova[1], Emilie Widemann[1], Laure Poirier[1], Yann Aubert[1], Laurence Miesch[2], Franck Pinot[1] and Rozenn Ménard[1]

*[1]IBMP-CNRS Université de Strasbourg, [2]LCOS UMR7177 CNRS Université de Strasbourg*

Jasmonates (JAs) are well-known regulators of plant defense responses to external cues and mediate also developmental processes like fertility. Since the demonstration that the conjugate jasmonoyl-isoleucine (JA-Ile) rather than jasmonic acid (JA) is the conserved hormonal signal, elucidation of JA-Ile turnover mechanisms has attracted interest.

We and others have characterized biochemically and genetically two JA-Ile catabolic pathways that are stress-inducible in leaves and developmentally regulated in flowers. The first pathway consists in Arabidopsis in a group of 3 co-regulated cytochromes P450 of the CYP94 subclade that define a two-step JA-Ile ω-oxidation process. CYP94B3 is the main enzyme catalyzing JA-Ile turnover/inactivation through hydroxylation upon mechanical leaf wounding. In contrast, upon fungal infection or in maturing stamens, the JA signatures in tissues reflect the predominant expression of CYP94C1. CYP94C1 exhibits peculiar properties as performs further oxidation to 12COOH-JA-Ile, a totally inactive derivative. All 3 enzymes oxidize JA-Ile and some less abundant JA-amino acid conjugates, and CYP94C1 additionally generates aldehyde intermediates. Unexpectedly, single or multiple mutations in CYP*94* genes, while increasing the amount and half-life of JA-Ile at the expense of hydroxy- and carboxy-derivatives, has negligible consequences on defense responses amplitude or antifungal resistance. This suggests that mechanisms may exist, including JAZ repressor hyperinduction, that desensitize signaling under high JA-Ile levels. In contrast, ectopic overexpression of CYP94B3 or CYP94C1 reduces JA-Ile levels and shuts down induced defenses and associated resistance.

The second pathway is defined by IAR3 and ILL6 amido-hydrolases that additionally contribute to hormone homeostasis by cleaving JA-Ile and 12OH-JA-Ile conjugates, providing an indirect route for the formation 12OH-JA and its derivatives. Catabolic pathways redefine a complex metabolic grid, where JA-Ile positions as a hub initiating the formation of many derivatives through oxidation and deconjugation. These metabolic circuits may reveal additional conversion routes with potential regulatory functions.

**4:00-5:30 pm Concurrent 2B: Hormone Transport**

**Functional screening of plant hormone transporters using modified yeast two-hybrid systems with receptor complexes**

Mitsunori Seo[1]

*[1]RIKEN Center for Sustainable Resource Science*

Studies have indicated that most plant hormones are mobile. However, it remains largely unknown how the transport of plant hormones is regulated. It has been reported that the receptors of plant hormones such as abscisic acid (ABA), gibberellin (GA) and jasmonoyl isoleucin (JA-Ile) interact with regulatory proteins in the hormone dependent manners. Thus, we usedyeast two-hybrid (Y2H) systems with the receptor complexes to look for proteins capable of transporting plant hormones. After screening of proteins that can promote interactions between the ABA receptor PYR/PYL/RCAR and PP2C protein phosphatases under low ABA concentrations, we identified a member of Arabidopsis NRT1/PTR FAMILY (NPF) proteins as an ABA importer. We subsequently found that some other members of NPF could transport ABA, GA and/or JA-Ile. Furthermore, we conducted a screening for GA transporters using the Y2H system with the GA receptor GID1a and the DELLA proteins GAI, and identified potential GA importers that belong to a transporter family other than NPF. We are now analyzing in vivo functions of the potential plant hormone transporters.

**NPF proteins are part of protein regulatory network involved in hormone-dependent nutrient sensing**

Benoît Lacombe[1]
[1]*Biochimie et Physiologie Moléculaire des Plantes, UMRCNRS/INRA/UM2/SupAgro, Institut de Biologie Intégrative des Plantes "Claude Grignon", Place Viala*

Members of the plant NITRATE TRANSPORTER 1/PEPTIDE TRANSPORTER (NRT1/PTR) family (NPF) display protein sequence homology with the SLC15/PepT/PTR/POT family of peptide transporters in animals. Compared to their animal and bacterial counterparts, these plant proteins transport a wide variety of substrates: nitrate, peptides, amino acids, dicarboxylates, glucosinolates, IAA, JA, GA and ABA. Using functional screens in heterologous systems, we have identified transporters in this family that are part of protein regulatory network involved in hormone dependent nutrient sensing in Arabidopsis. Other approaches initiated to identify other component of nitrate-hormone crosstalks will be presented.

**Long-distance transport of endogenous gibberellins in *Arabidopsis***

Thomas Regnault[1,2], Jean-Michel Davière[1], Michael Wild[1,3], Lali Sakvarelidze-Achard[1], Dimitri Heintz[1], Esther Carrera[4], Isabel Lopez Diaz[4], Fan Gong[5,6], Peter Hedden[5] and Patrick Achard[1].
[1]*Institut de biologie moléculaire des plantes, CNRS, Université de Strasbourg,* [2]*Department of Plant Systems Biology,* [3]*University of Freiburg,* [4]*Instituto de Biología Molecular y Celular de Plantas, CSIC-UPV,* [5]*Rothamsted Research, Harpenden,* [6]*Home Office Science – Centre for Applied Science and Technology*

Plant hormones are small signaling compounds, often present at very low concentrations, which act either locally or near the site of synthesis, or in distant tissues. Gibberellins (GAs) are phytohormones controlling major aspects of plant growth and development. Although previous studies suggested the existence of a transport of GAs in plants, the nature and properties associated with this transport were unknown. By mixing old-style grafting with modern molecular genetics in *Arabidopsis*, we show that the $GA_{12}$ precursor, although biologically inactive, is the chemical form of GA undergoing long- distance transport across plant organs. We propose that long-distance transport of $GA_{12}$ across plant organs enables plants to adapt their growth and development in response to both endogenous and environmental inputs.

**The over expression of the strigolactone transporter PDR1 as a tool to improve plant growth on phosphate poor soils**

Guo-Wei Liu[1], Johannes Pfeifer[2], Marina Stirneman[1], Christian Gübeli[1], Joëlle Schlapfer[3], Achim Walter[2], Enrico Martinoia[1], and Lorenzo Borghi[1]
[1]*University of Zurich,* [2]*ETH Zürich,* [3]*Carnegie Institution for Science*

The phytohormone strigolactone (SL) was discovered 50 years ago as germination stimulant for parasitic weeds. In the last years we learned that SLs are carotenoid derivatives playing several additional roles. In the interaction between plants and environment, SLs induce hyphal branching of mycorrhizal fungi, thus promoting the first steps of root mycorrhization. In plants, SLs regulate shoot and root architecture, such as the development of lateral organs (1).
As for animal hormones, plant hormones are often transported from their site of synthesis to their site of action, even over long distances (2). We investigate how SLs are transported from the root to the soil as well as to the shoot, as suggested by previous experiments (3). The only up-to-date characterized SL transporter, the ABC protein PDR1, is required for SL exudation to the soil and deliver to the aboveground part of the plant (4). PDR1 is asymmetrically localized in root cortex cells, thus suggesting that polar SL transport is necessary for SL function (5). *pdr1* mutants are less efficient than wildtype in establishing mycorrhization and have increased shoot lateral branching. On the contrary, plants over- expressing PDR1 (PDR1 OE) have a pronounced apical dominance and their root exudates strongly promote the germination of parasitic weeds. Both these

phenotypes are related to de-regulated SL transport/exudation.
We report here the effects of PDR1 OE on plant biomass production, root development and mycorrhization in two Solanaceae, *Petunia hybrida* and *Nicotiana benthamiana*. PDR1 OE plants produce more biomass on nutrient deprived soils, compared to wildtype, due to higher/faster mycorrhization levels and to dramatic changes in root cell identity and root architecture. As resulted from x-ray tomography analyses on petunia roots, PDR1 OE promotes lateral root development, root hair elongation and the amount of specialized cortex cells called hypodermal passage cells (HPCs). HPCs are exodermal, non-suberized cells and are the exclusive entry points for mycorrhizal fungi. Additionally, HPCs likely regulate the uptake of several ions from the soil to the root, as they lack the hydrophobic suberin layer present in neighboring hypodermal cells. Our first results indicate that SL, in crosstalk with other phytohormones, regulates the identity and/or maintenance of HPCs, thus integrating soil nutrient uptake with root/shoot architecture and plant biomass production.


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**Intracellular auxin gradient is essential for the tip growth of a protonemal cell in the moss, *Physcomitrella patens***


Kousuke Fukui[1], Akihiro Oochi[1], Naoki Takeuchi[1], Hiroyasu Motose[2], Takashi Aoyama[3], Tomomichi Fujita[4], and Ken-ichiro Hayashi[4]

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The polar auxin transport produces an asymmetric auxin gradient and plays an essential role in spatiotemporal control of plant development. Auxin efflux carrier proteins, PIN mainly modulate the direction and rate of auxin movement. Several PIN family proteins coordinately establish the local auxin maxima at root tip and then consequently determine the position of a quiescent center in the root meristem in vascular plants.
Recent reports demonstrated that auxin transport machinery is functionally conserved in an early diverging land plant lineage, such as the moss, *Physcomitrella patens*. In *P. patens*, PIN carrier proteins are localized at plasma membrane in the apical tip of a protonemal cell, a tip-growing filamentous cell of the moss. Auxin promotes chloronema to caulonema transition during protonemal development. Disruption of SCF (TIR1) auxin signaling in *P. patens* by RNAi and auxin antagonist repressed the transition to caulonema. To investigate the physiological function of auxin transport in the moss, we applied chemical biology approach using competitive-type auxin transport inhibitor and fluorescent auxin analogs into auxin transport system. In consistent with the localization of PIN at the apical tip of a protonemal cell, our results indicate auxin transport system plays a crucial regulatory role for the apical tip growth of a protonemal cell in *P. patens*.

**JEFF1 and JEFF2 facilitate jasmonate efflux and affect the wound response in Ararbidopsis thaliana.**
Sophie Lambertz[1], Morten Egevang Jørgensen[1], Meike Burow[1], Christopher Crocoll[1], and Hussam H. Nour-Eldin[1]
[1]*DynaMo Center, Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen*

Plants critically depend on defense mechanisms that are systemically induced upon herbivore attack. The systemic induction initially relies on electrical wound-signals transmitted from the site of attack to the whole plant body. In distal tissues electrical signals are presumably decoded into a chemical jasmonate borne signal, which is detected as dramatically increased accumulation of jasmonates. This decoding takes place in so-called xylem-contact cells, where either precursors are released or jasmonates are *de novo* synthesized. Propagation of the jasmonate signal from xylem-contact cells to extravascular cells were proposed to proceed through the physical movement of jasmonate. Such movement would require the activity of jasmonate export. However, no jasmonate exporters have been identified yet.
Here, we screened the NPF family for jasmonate export activity and identified, three related transport proteins that facilitate efficient jasmonate efflux (*JEFF1, 2* and *3)* in the *Xenopus laevis* heterologous expression system. Localization by promoter GUS reporter constructs show that *JEFF1* and *JEFF2* are expressed in vascular tissue of *Arabiopsis thaliana* aerial and root tissue. In preliminary wounding assays of seedlings of the *jeff1jeff2* dko mutant, a lower JAZ10 transcript induction was detected.
Additionally, bioassays with *Spodoptora littoralis* indicate a reduction in resistance of *jeff* kos compared to WT plants. This indicates that export of jasmonate mediated by *JEFF1* and *JEFF2* may be a critical component of the jasmonate response associated with plant-herbivore interactions.

**4:00-5:30 pm Concurrent 2C: Hormone Genomics**

**Harnessing the power of molecular evolutionary analysis to understand strigolactone signaling**
David Nelson[1]
[1]*Department of Genetics, University of Georgia*

Modern DNA sequencing technology has granted unprecedented access to the genetic diversity found in non-model organisms, which has been sifted by evolutionary selection. This wealth of information can be used to investigate hormone signaling mechanisms in a guided manner that is more efficient than random mutagenesis. Parasitic weeds in the Orobanchaceae family use strigolactones as germination stimulants that indicate the presence of a nearby host root. We investigated the basis of host perception in the Orobanchaceae through an evolutionary analysis of karrikin and strigolactone receptor sequences, coupled with homology modeling and cross-species complementation. We found evidence that in parasite genomes, duplication of the karrikin receptor *KAI2* was followed by the evolution of strigolactone perception amongst a clade of fast-evolving *KAI2* paralogs. We extended our analysis to predict which genes found in the basal land plant *Physcomitrella patens* might be involved in strigolactone perception. We are now using several in depth comparisons of functionally diverse *KAI2* paralogs as a basis for reconstructing the evolution of different ligand specificities.

**Efficient mapping of genome-wide regulatory elements for biological insights**

Shao-shan Carol Huang[1]
[1]*Joe Ecker Lab, Salk Institute*

We developed a high-throughput sequencing assay for rapid transcription factor binding site (TFBS) discovery, DNA affinity purification sequencing (DAP-seq), that uses *in vitro* prepared transcription factors (TFs) to capture native genomic DNA. We applied DAP- seq to 1,812 *Arabidopsis thaliana* TFs to resolve motifs for 529 factors and genome-wide enrichment maps for 349 factors. Cumulatively, the ~2.7 million experimentally- determined TFBSs captured the Arabidopsis cistrome and predicted thousands of TF target genes enriched for known and novel functions. Notably, DAP-seq target genes for many well-characterized hormone related TFs were enriched for Gene Ontology terms consistent with their known functions. Comparison of DAP-seq and ChIP-seq datasets showed that DAP-seq peaks predicted *in vivo* TF binding better than motif inference, potentially due to the ability of the assay to directly capture the impact of primary sequence and DNA methylation on binding affinities at individual TFBS. As a demonstration of the importance of genomic context, we showed that closely spaced motifs significantly affected TF binding by developing a model for cooperative auxin response factor (ARF) homodimer binding to complex motif repeats. Overall, DAP-seq enables rapid development of base-resolution cistrome atlases for a wide-array of applications for eukaryotic genomes.

**Chemical Genomics To Unravel Auxin Perception Controlling Arabidopsis Seedling Development**

Thomas Vain[1], Noel Ferro[2], Deepak Kumar Barange[3], Qian Ma[1], Mattias Thelander[1], Barbora Pařízková[4], Ondřej Novák[4], Siamsa M. Doyle[1], Alexandre Ismail[5], Per Anders Enquist[3], Adeline Rigal[1], Malgorzata Langowska[1], Yi Zhang[6], Karin Ljung[1], Judy Callis[7], Fredrik Almqvist[3], Mark Estelle[6], Laurens Pauwels[8] and Stéphanie Robert[1]
[1]*Swedish University of Agricultural Sciences,* [2]*Bonn University,* [3]*Umeå University,* [4]*Palacký University,* [5]*Sup'Biotech, IONIS Education Group,* [6]*University of California San Diego,* [7]*University of California Davis,* [8]*ViB-PSB*

Auxin phytohormones control most aspects of plant development through a complex and interconnected signaling network. In the presence of auxin, AUXIN/INDOLE-3- ACETIC ACID (Aux/IAA) transcriptional repressors are targeted for degradation by the SKP1-CULLIN1-F-BOX (SCF) ubiquitin-protein ligases containing TRANSPORT INHIBITOR RESISTANT 1/AUXIN SIGNALING F-BOX (TIR1/AFB). CULLIN1-neddylation is required for $SCF^{TIR1/AFB}$ functionality as exemplified by mutants deficient in the NEDD8-activating enzyme subunit AUXIN- RESISTANT 1 (AXR1). Redundancy within the auxin perception machinery hinders chemical genomics approaches to the identification of auxin analogs. Here, we report four small molecules named DEVELOPMENTAL REGULATORS (DRs) requiring AXR1 and $SCF^{TIR1/AFB}$ to modulate plant development. Three DR molecules trigger selective auxin responses at transcriptional and morphological levels.

**The root-derived bps signal induces ABA-dependent and ABA-independent changes in gene expression.**

Dong-Keun Lee[1,2], David L. Parrott[1], Eiji Nambara[3], and Leslie E. Sieburth[1]
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The Arabidopsis *bypass1* (*bps1*) mutant shows a severe seedling growth arrest phenotype. This growth arrest is a response to a novel mobile metabolite that is over-produced in its roots, and that is also sufficient to arrest growth of a wild-type plant. Our goal is to determine whether the *bps* signal is a plant hormone that has not been previously described. One of our approaches is to characterize responses of wild-type tissues to the *bps* signal using transient micrografts. We place a wild- type scion and *bps1* or wild-type (control) rootstock in a grafting collar, separated by an agarose block. Wild-type scions show robust responses to the *bps* signal within 24h. Evidence for the *bps* signal controlling gene expression came from using scions carrying GUS markers

(*CYCB1:1::GUS* and the stem-cell marker *pWUS::GUS*). To characterize the genome---wide responses to the *bps* signal, we carried out RNAseq on wild-type scions grafted to *bps1* or wild-type roots. This analysis identified 353 up-regulated and 81 down-regulated genes. GO analysis showed over-representation of water deprivation, salt stress, and ABA response genes. Although *bps1* double mutants that abrogate ABA synthesis are phenotypically identical to *bps1*, we considered that ABA might be mediating a *bps* signal---initiated physiological response. To test this, we carried out RNAseq on scions from transient micrografts that coupled *aba3* mutant scions with either *aba3* or *bps1 aba3* roots. This analysis revealed a core set of genes that respond to the *bps* signal independently of ABA, a second set of genes regulated through *bps* signal-ABA cross talk, and a third large set of ABA-specific genes. These findings are consistent with the *bps* signal being a novel plant hormone that functions upstream of ABA production, and that also controls development through ABA-independent regulation of gene expression.

**Internal and External Signals Controlling Radial Expansion of Root Systems**

Sascha Waidmann[1], Michel Ruiz Rosquete[1], and Jürgen Kleine-Vehn[1]
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As soon as a seed germinates, plant growth relates to gravity to ensure that the root penetrates the soil and the shoot expands aerially. Mechanisms of positive and negative orthogravitropism of primary roots and shoots are relatively well understood. In contrast, lateral organs show more complex growth behavior, which largely remains enigmatic. Root growth towards gravity is an important trait that ensures sessile plants to anchor and to cover the soil. Lateral roots (LRs) are important to increase the root soil surface and allow for radial expansion of the root system (plagiotropism). For such a radial exploration of the substrate, LRs seemingly suppress positive gravitropic growth and show a defined gravitropic set-point angle (GSA) (Ruiz Rosquete et al., 2013). We here illustrate that plants from diverse geographical origin have differences in GSA-derived root system architecture, suggesting importance of this trait for habitat compatibility. We quantitatively determined GSA of emerged lateral roots in naturally occurring *Arabidopsis* accession lines and have used genome-wide association (GWA) techniques to reveal novel molecular components involved in GSA determination. Based on this genetic screen, we provide molecular evidence that cytokinin metabolism plays an important role in the GSA establishment in lateral roots. Mechanistically, cytokinin counteracts the auxin transport-dependent angular growth of lateral roots, thereby promoting radial expansion of the root systems. We are currently investigating whether this mechanism guides to integrate cytokinin-reliant shoot apical meristem activity with auxin transport-dependent regulation of directional lateral root growth.

**Identification of gibberellin signaling components in cold stress**

Ourania Lantzouni[1], Alexandre Magalhães[1], Angela Alkofer[1], Pascal Falter-Braun[1], and Claus Schwechheimer[1]
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The phytohormone gibberellin (GA) is implicated in many agronomically important processes including plant responses to abiotic stresses such as cold. Exposure of *Arabidopsis* to low temperature results in growth inhibition and transcriptional changes that lead to cold acclimation. Exposure to cold temperature also leads to a reduction of GA hormone levels and the stabilization of DELLA proteins, critical growth repressors of the GA pathway. DELLA repressors interfere with the activity of a range of pathway-specific transcription factors through protein-protein interactions. We aim to understand how GA and DELLAs regulate the cold-stress response by identifying downstream DELLA targets in this pathway. To this end, we looked into the contribution of DELLA-dependent transcriptional changes in cold stress using RNA-Seq. We discovered that around 9 % of the cold-induced transcriptional changes are differentially expressed during

concomitant GA application. Interestingly, application of GA affected a largely different gene set upon cold stress than application of GA to ambient temperature-grown seedlings. The later suggests that GA and hence the control of transcription by DELLAs depends on the different dynamics of protein- protein interactions in ambient temperature versus cold stress. To identify DELLA-regulated transcription factors that may operate during cold stress, we performed a yeast two-hybrid screen with a collection of 2000 Arabidopsis transcription factors where we identified more than 200 DELLA interactors for the two DELLAs, RGA and GAI. These included 32 DELLA- regulated transcription factors that were also differentially regulated in the above described transcriptomics experiment. Our current focus is on the elucidation of the role of DELLA interaction with candidate transcription factors in mediating cold stress-specific GA responses.

Keywords: Arabidopsis, DELLA, cold stress, gibberellin

**Thursday, June 23, 2016**

**9:00-10:30 am Plenary III: Hormones & Environment**

**DELLA-dependent salt stress tolerance network**

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DELLAs are central regulators of gibberellin (GA) signaling, which work at repression of GA-regulated gene expression. These GRAS proteins bind the DNA recognition domain of many different families of transcription factors, and sequester these regulators into an inactive complex unable to bind to DNA[1]. Physical interaction of DELLAs with light-regulated PHYTOCHROME INTERACTING FACTOR 3 and 4 (PIF3 and PIF4) and the BR-signaling BRI1-EMS-SUPPRESSOR1/ BRASSINAZOLE-RESISTANT1 (BES1/BZR1) factors suppresses plant growth, by blocking PIFs and BES1/BZR1 dependent co-activation of multiple cell wall-remodeling and auxin- signaling related genes, required for cell elongation[2-4]. Growth restraint imposed by the DELLAs correlates with increased tolerance to drought, salt and cold stress. Although growth inhibition was accepted to contribute to this response, more recent studies showed that DELLAs play an active role in stress survival, by activating the expression of genes involved in ABA-signaling and ROS protection[5,7]. To gain molecular insight into this DELLA-dependent stress activation pathway we screened a collection of TF- overexpressor lines for their ability to confer increased tolerance to salt stress, in the presence of GAs. Several regulators previously reported to orchestrate abiotic stress responses to, were in this way identified. Remarkably, nearly one third of these factors were observed to physically interact with the DELLAs, and these repressors bind in many of these factors a different region than the DNA recognition motif, in agreement with DELLAs activating their downstream targets. Moreover, mapping of the DELLA interaction domain showed that interaction did not involve the LHR1 heptad repeat, implicated in PIFs and BES1/BZR1 interaction, but the DELLA domain or C-terminal end. This indicates that it should be feasible identifying DELLA mutations that impair PIF and BES1/BZR1 interaction, but not affect binding to these stress-related factors. We propose that expression of stable forms of the DELLAs carrying these mutations will confer increased tolerance to drought and salt stress, without a negative effect in plant growth. Characterization of these lines will be instrumental to improve our understanding of the DELLA-dependent stress pathways, and in plant breeding, useful to select new genotypes in which stress-tolerance is uncoupled from growth restraint.

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**Beyond the Green Revolution: new approaches for improving nitrogen use efficiency and grain yield in rice**

Qian Liu[1], Xiangbin Chen[1], Hongying Sun[1], Kun Wu[1], Shuansuo Wang[1], and Xiangdong Fu[1]
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Nitrogen fertilization is essential to increase grain yield, whereas it also promotes stem and leaf elongation and makes crop plants more susceptible to lodging, resulting in yield lose. The Green Revolution of the second half of the 20th century, which was based on the adoption of semi-dwarf cereals which had an increased harvest index, was responsible for worldwide crop yield increases and enhanced global food security. However, introduction of semidwarf genes *sd1* and *Rht* into rice and wheat caused the reduction of panicle (or ear) branching and nitrogen uptake capacity, and increase in grain yields required significant increases in nitrogen fertilization levels, which in turn resulted in what are now well documented deleterious impacts on the environment. To uncover the role of the DELLA protein in control of nitrogen uptake and assimilation, we performed a yeast two-hybrid screening to identify DELLA-interacting proteins. In the further experiments we showed an interactive network for gibberellin control of nitrogen acquisition and nitrogen-mediated growth responses. In addition, we also show that a rice major QTL, which act through the determination of both panicle architecture and nitrogen growth responses. The different *DEP1* alleles confer different nitrogen- mediated growth responses. Importantly, *dep1* plants are not only semidwarf but also had increased nitrogen-use efficiency. The DEP1 protein physically interacts with Gα (RGA1), and Gβ (RGB1) and reduced RGA1 or enhanced RGB1 activity represses nitrogen-mediated growth. Thus, the modulation of both GA and G-protein signalling represents a strategy to simultaneously improve nitrogen use efficiency and grain yield.

**2:00-3:35 pm Concurrent 3A: Chemical Biology**

**Chemical regulation of plant hormone functions and their cross talk: SL, GA, BL and Et**

Tadao Asami[1]
[1]*Graduate School of Agricultural and Life Sciences, The University of Tokyo Department of Biochemistry, King Abdulaziz University, Jeddah, Saudi Arabia*

Plant hormones are biosynthesized and perceived by their receptors and then elicit their activity to regulate plant life cycles. Various steps in the life cycles of plants can be modulated by each of hormones, sometimes in a synergistic fashion, suggesting physiological redundancy and/or crosstalk between the different pathways. As small molecules can be used to unravel these plant hormone functions, many chemicals that can dissect plant hormone functions have been developed and accelerate our understanding of plant hormone signaling. In this context, we developed several chemical regulators for plant hormone functions, such as biosynthesis inhibitors, catabolism inhibitors, receptor inhibitors and mimics. Here, we will talk about the development and/or characterization of known or new chemical regulators mainly for GA and SL from the viewpoint of the reduction of crop damage by root parasitic weeds.

1. AC94377 is a GID1 agonist that preferentially binds a specific GID1 to activate the GA signal in Arabidopsis.
2. Debranones are SL functionally selective mimics.
3. KUT15 is an ethylene mimic that partially activates ethylene signal.

These chemicals can control damage by root parasitic weed such as Striga.

- AC94377 can reduce SL production in rice.
- Debranones reduce SL production maybe by feedback regulation.
- KUT15 can induce suicidal germination of Striga and can reduce the seedbank of Striga.

**A small-molecule approach to identify chemical activators of brassinosteroid signaling**

E. (Jenny) Russinova[1,2]
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Clathrin-mediated endocytosis (CME) is a major pathway for the uptake of membrane proteins, lipids, and extracellular molecules into plant cells and is of vital importance for the plant development as multiple cellular processes, including nutrient uptake, signal transduction, and plant- microbe interactions, require CME. Brassinosteroid (BR) hormones are perceived at the cell surface by the constitutively endocytosed receptor BRASSINOSTEROID INSENSITIVE1 (BRI1). Inhibition of CME of BRI1 prevented receptor desensitization and had a positive effect on brassinosteroid signaling suggesting that signaling responses can be modulate via CME. However, the genetic inhibition of CME is detrimental for plant growth. Chemical inhibitors of CME are an attractive alternative, but despite the available extensive structural and biochemical knowledge about CME in mammalian cells, the development of chemical tools to interfere with this process is still limited in all systems. Our study is focused on the identification and characterization of novel compounds that target CME and activate brassinosteroid signaling. The development of novel inhibitors will contribute to better molecular and functional dissection of CME in plants and to an increased understanding of how CME controls receptor-mediated signaling.

**Mechanism of strigolactone reception through pea receptorstudies**

Alexandre de Saint Germain[1,2,3] , Guillaume Clavé[4], Marie-Ange Badet-Denisot[4], Jean-Pierre Le Caer[4], Joanne Chory[2,3], Catherine Rameau[1] and François-Didier Boyer[1,4]
*[1]Institut Jean-Pierre Bourgin, INRA, AgroParisTech, CNRS, Université Paris-Saclay, [2]Howard Hughes Medical Institute, [3]Plant Biology Laboratory, The Salk Institute for Biological Studies, [4]Institut de Chimie des Substances Naturelles, CNRS UPR2301, Univ. Paris-Sud, Université Paris-Saclay*

Strigolactone plant hormones control plant architecture and are signals in soil to parasitic weeds (*Striga* and *Orobanche*) and symbiotic arbuscular mycorrhizal fungi. They contain an ABC tricyclic lactone connected to a butenolide group, the D ring. The D14 strigolactone receptor belongs to the superfamily of α/β-hydrolases and hydrolyzes strigolactone in ABC and D parts. In this presentation, we will present our results concerning the characterization of the binding and catalytic functions of RMS3, the pea ortholog of rice D14 strigolactone receptor. Using novel profluorescent probes with strigolactone-like bioactivity, we proposed a hypothesis that explains the apparent low enzymatic rate of RMS3. The formation of a covalent RMS3/D-ring complex, essential for bioactivity, was identified by mass spectrometry data. These results reveal an undescribed mechanism of plant hormone reception where the receptor itself performs an irreversible enzymatic reaction to generate its own D ring ligand.

**A forward genetic screen on chemicals that disrupt the actin cytoskeleton uncovers a novel regulator of auxin efflux carrier trafficking in Arabidopsis**

J. Alan Sparks[1], Taegun Kwon[1], and Elison B. Blancaflor[1]
*Plant Biology Division, The Samuel Roberts Noble Foundation*

To discover new proteins that function in actin–dependent cellular processes in plants, we isolated *Arabidopsis thaliana* mutants that were resistant or hypersensitive to latrunculin B (LatB), a potent chemical inhibitor of the actin cytoskeleton. We isolated 3 non-allelic mutants that had enhanced sensitivity to, and 4 non-allelic mutants that were tolerant to the growth inhibitory effects of LatB. One recessive mutant, *hypersensitive to latrunculin B 1*(*hlb1*), was disrupted in a gene (*AT5G41950*) encoding a tetratricopeptide repeat (TPR) domain-containing 565 amino acid protein of unknown function. Nanomolar concentrations of LatB induced more profound alterations of seedling growth and F-actin organization in *hlb1* compared to wild type. Surprisingly, *hlb1* was also hypersensitive to the actin stabilizing chemical Jasplakinolide. In addition to its heightened sensitivity to LatB, *hlb1* had aberrant root hair shape and mild primary root growth defects. Further, *hlb1* had reduced vegetative growth under short day conditions. A functional HLB1-GFP fusion colocalized with *trans*-Golgi Network (TGN)/early endosome (EE) markers through its conserved C-terminal domain. Recycling of the auxin efflux carrier, PIN- formed 2 (PIN2) to the plasma-membrane was disrupted in *hlb1*. Co-immunoprecipitation identified the TGN/EE-localized Brefeldin A (BFA)-visualized endocytic trafficking defective 1 (BEN1) as a putative HLB1 interactor. Interestingly, *ben1* mutants were hypersensitive to LatB to the same extent as *hlb1* mutants. Genetic interaction studies and live imaging of HLB1-GFP in the *ben1* mutant background indicate that BEN1 is crucial for HLB1 localization to the TGN/EE. Based on these data, we propose that HLB1 together with BEN1 form a complex with actin to modulate trafficking of PIN2 at the intersection of the exocytic and endocytic pathways. Taken together, our results demonstrate the feasibility of forward genetic screens on actin-disrupting chemicals to uncover novel protein regulators that mediate interactions between the cytoskeleton and endomembrane trafficking (Supported by NASA grant NNX12AM94G).

**Chemical screening uncovers an antagonist for the strigolactone receptor HTL**

Duncan Holbrook-Smith[1], Shigeo Toh[2], and Peter McCourt[1]
*[1]University of Toronto, [2]Nagoya University*

*Striga* spp. (Witchweed) is an obligate parasitic plant that attaches to host roots to deplete them of nutrients. In Sub-Saharan Africa, the most destructive *Striga* species, *S. hermonthica*, parasitizes major food crops affecting two-thirds of the arable land and over 100 million people. One potential weakness in the *Striga* infection process is the way it senses the presence of a host crop. *Striga* only germinates in the presence of the plant hormone, strigolactone, which exudes from a host root. Hence small molecules that perturb strigolactone signalling may be useful tools to disrupt the *Striga* lifecycle. Here we developed a chemical screen to suppress strigolactone signalling in the model plant Arabidopsis. One compound, Soporidine, specifically inhibits a *S. hermonthica* strigolactone receptor and consistent with this, Soporidine inhibits germination of this parasite. This suggests strigolactone-based screens using Arabidopsis may be useful in identifying lead compounds to combat *Striga* infestations.

**Design and synthesis of fluorescently labeled 6-substituted purine derivatives as markers of cytokinin perception**

Karolina Kubiasová[2], Václav Mik[1], Martin Hönig[1], Jaroslav Nisler[1], Ondřej Plíhal[1], Lukáš Spíchal[1], Karel Doležal[1], Miroslav Strnad[1] and Lucie Plíhalová[1]

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Cytokinins and their synthetic derivatives play crucial role in the regulation of plant growth. Therefore, it is very important to understand and visualise the process of cytokinin perception to the cells. We selected isopentenyladenine and benzylaminopurine molecules as an easily obtainable and well substitutable model compounds and we accompanied the molecules with various fluorescent probes such as dansyl chloride, fluorescein (FC), rhodamine B, coumarine, 7-(diethylamino)coumarine-3-carboxylic acid (7-DEAC) and others attached on 2 or 6-carbon spacers at C2 or N9 atoms of the purine moiety. Selected representatives of novel fluorescently labeled molecules maintained cytokinin activity and some of them were able to trigger signaling pathway *via* cytokin-sensitive receptors AHK3 and CRE/AHK4 from *Arabidopsis thaliana* and/or *via* ZmHK1 and ZmHK3a from *Zea mays*. Although structural changes in several cytokinin derivatives led to the loss of the ability to initiate the signal transduction through the receptors studied, some derivatives were still able to interact with the receptor binding site and partially blocked binding of radioactive labelled competitor $^{3}$HtZ.

**2:00-3:35 pm Concurrent 3B: Light Responses**

**PhyB inhibits negative hypocotyl gravitropism non-cell autonomously**

Giltsu Choi[1]

[1]*Department of Biological Sciences, KAIST*

Seedling hypocotyls display negative gravitropism in the dark but agravitropism inthe light. Previous works showed that the *pif quadruple* (*pifQ*) mutant lacking four members of PHYTOCHROME-INTERACTING FACTORS (PIFs) are agravitropic in the dark and the expression of PIF in endodermis is sufficient to restore the gravitropism in the *pifQ* mutant. Since phytochromes are known to induce light responses by inhibiting PIF transcription factors and COP1-SPA ubiquitin E3 ligase complex in the nucleus, we examined if phyB inhibits hypocotyl negativegravitropism in the endodermis cell autonomously. We found that the expression of phyB by epidermis-specific promoters rescue all *phyB* mutant phenotypes including hypocotyl negative gravitropism, whereas the expression of phyB by endodermis-specific promoter does not rescue. Epidermal phyB induces the degradation of endodermal PIFs in response to red light, leading to global gene expression pattern similar to one induced by phyB driven by its own promoter. Our results imply that epidermal phyB generates yet unidentified mobile signals that travel to endodermis where it promotes the degradation of PIFs to inhibit hypocotyl negative gravitropism.

**Shade Avoidance Requires Multiple Hormone Signaling Pathways**

Kazunari Nozue[1], Susan Bush[1], Leonela Carriedo[1], Patricia Mueller-Moule[1], Neelima Sinha[1] and Julin Maloof[1]

[1]*University of California, Davis*

Many plants have a sophisticated suite of responses to shade from neighboring plants that enable them to compete with their neighbors for photosynthetic light. Known as the shade avoidance response, the response to neighbor shade can include increased stem elongation, altered branching, and early flowering. Shade avoidance responses come at the expense of resource allocation to fruit and seed and thus reduce agronomic yield. I will

cover three topics illustrating the importance of hormones to shade avoidance. First, while auxin has long been implicated in shade avoidance, the mechanisms for increased auxin under shade have not been fully elucidated. We have found that the *YUCCA* auxin biosynthetic genes are absolutely required for shade avoidance. Second, we have found that variation in auxin signaling may underlie quantitative trait loci (QTL) for shade avoidance variation in tomato. Lastly, I will discuss the results of phenotypic and transcriptional studies that have revealed new connections between, jasmonic acid, salicylic acid, and shade avoidance.

**Effects of elevated ambient pressure and temperature on rates of net photosynthesis and dark respiration**

Shinya Sawada[1], Motoki Yonekura[1], Hiroyuki Takeishi[1], Jun hayashi[1,3], Takashi Machimura[1,3], Yoshihiro Koshino-Kimura[2], Akio Kobayashi[2,3], and Fumiteru Akamatsu[1,3]
*[1]Graduate school of Engineering, Osaka University, [2]The Institute of Scientific and Industrial Research, Osaka University, [3]On-site Research Center for Sustainability Design, Graduate school of Engineering, Osaka University*

This study investigates effects of environmental conditions on net photosynthesis by using artificially controlled environment. Especially, the effects of elevated ambient pressure were focused. This study discussed about the CO2-exchange rates of aseptic-medium-cultured plants, *Arabidopsis thaliana*, which were observed under the conditions of elevated ambient pressures and controlled temperatures. The pressure chambers, which were made of acrylic and stainless, were used to enclose plants under elevated ambient pressure. Fluorescent lights and LED were used as light sources, PPFD (photosynthetic photon flux density) was controlled by the ambient range from 0 μmol $m^{-2}s^{-1}$ (dark period) to 200 μmol $m^{-2}s^{-1}$ (light period). The pressure was regulated to 0.1, 0.2 and 0.3 MPa. Compressed-air ($CO_2$; 400ppm, $O_2$; 21%,) was used for the experiments. The photosynthetic rate was measured by using an IRGA in the temperature range of 12°C - 36°C at an interval of 4°C.
Results show that, while the net photosynthetic rate was increased, the rate of dark respiration fluctuated little at all measurement temperatures under the elevated ambient pressures. Also, rates of net photosynthesis and dark respiration almost always increased with the rise of temperature. In exceptional condition that the temperature increased from 32°C to 36°C, they decreased with the rise of temperature. This result indicated that the temperature at the peak of net photosynthesis fell to 32°C under elevated ambient pressure. The optimal temperature of gross photosynthesis theoretically increases with raised $CO_2$ partial pressure, whereas this experiment suggests that raised $CO_2$ and $O_2$ partial pressures may shift the optimal temperature down by the different temperature dependency of carboxylation and oxygenation.

**The Interaction of Light and Gibberellin in the Control of Wheat Architecture**

Bethany Ellis [1,2], Peter Hedden[1], Steve Thomas[1], Andy Phillips[1] and Matthew Terry[2]
*[1] Rothamsted Research, [2] Centre for Biological Sciences, University of Southampton*

DELLA proteins are repressors of plant height that act in the gibberellin-response pathway. The green revolution saw DELLA mutations introduced into wheat, causing a dwarf phenotype that reduced lodging and increased grain biomass, greatly improving yields. Phytochrome Interacting Factors (PIFs) are transcription factors that mediate light responses in plants, including promotion of hypocotyl elongation. In Arabidopsis, DELLAs repress the transcriptional activity of PIFs through a direct physical interaction. This highlights a role for PIFs in regulating growth in response to gibberellin. Furthermore, a recent paper indicated that a rice PIF homologue, OsPIL1, is an important regulator of stem elongation[1]. This suggests that *PIF* expression is a target for modifying stem height in wheat, with the potential benefit of increasing yields.
Wheat contains one DELLA, RHT-1, whereas three OsPIL1 orthologues were identified using bioinformatics; we have named these TaPIL1, TaPIL2 and TaPIL3. TaPIL1 is the most closely related to OsPIL1 and TaPIL2 the least, with a protein sequence-identity of 69% and 32.3% respectively. The interaction between RHT---1 and TaPIL1

was investigated using a yeast two-hybrid assay, revealing a possible interaction between these proteins. A yeast two-hybrid library is being used to screen for RHT-1 interactors in the wheat stem, the latest screen identified many clones encoding potential interactors which are being characterised. The role of the TaPILs in regulating stem elongation and light response is being analysed by altering expression levels. RNAi and TILLING lines are being produced to assess the effect of reduction or knockout of TaPIL1 expression. Overexpression of both TaPIL1 and the rice OsPIL1 will be used to assess effect on plant stature. Through investigating the interaction of RHT-1 and wheat PIFs, and their implications for architecture and light responses, we aim to increase our understanding of gibberellin signalling and identify new potential targets for improving wheat yields.

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**PHYA and PHYB regulate adventitious rooting in response to dark-light transitions in Arabidopsis seedlings**

Molly Kreiser[1], Jerry Cohen[1], and Gary Gardner[1]
[1]*University of Minnesota*

Adventitious rooting can be induced without exogenous hormones in some species by transitioning plants from dark to light environments. We are investigating the role of light quality, plant photoreceptors, and auxin levels in this process. Etiolated wild type Arabidopsis seedlings exposed to red, white, or blue light for one week produced significantly more adventitious roots than seedlings that were kept in continuous darkness or exposed to far red light only. Phytochrome B (phyB) mutants produced significantly more adventitious roots than wild type in response to red or white light treatments, but also produced very few adventitious roots under dark conditions. Phytochrome A (phyA) mutants and phyAphyB double mutants produced significantly fewer roots than wild type under all light treatments tested. These results suggest that PHYA and PHYB regulate adventitious rooting in response to dark-light transitions, with PHYA being required for adventitious root formation and PHYB functioning as an inhibitor. We are currently investigating whether differences in auxin levels or metabolism may contribute to differences in root formation among wild type, phyA, and phyB seedlings using highly sensitive GC-MS/MS analysis. These findings have implications for better understanding and potentially improving adventitious rooting in horticulturally important species, which is often a bottleneck to propagation and production.

**2:00-3:30 pm Concurrent 3C: Gasotransmitters**

**Function and evolution of oxygen and nitric oxide sensing through the N-end rule pathway**

Michael Holdsworth[1]
[1]*University of Nottingham*

Our recent work uncovered the simple biochemical mechanism that plants use to simultaneously sense oxygen and nitric oxide (NO), through the N-end rule pathway of ubiquitin-mediated proteolysis [1,2]. The AP2-domain ERFVII transcription factors were shown to be substrates of this pathway, with conditional stability based on the oxidation status of Cys-2 providing a homeostatic response mechanism to changes in oxygen and NO levels. This mechanism is used by plants to measure important ecological cues to protect the stem cell niche and enhance survival [3]. I will discuss our attempts to understand how plants use this unique and evolutionarily ancient branch of the Ubiquitin Proteasome System to perceive environmental change through sensing fluctuations in both gases. I will also provide evidence that this mechanism includes not only ERFVII substrates, but a cohort of other unrelated proteins, allowing a rapid response to changes in gas levels at the proteome level. Although the N-end rule pathway is ancient, evolution of

ERFVIIs and other substrates occurred relatively recently in the land plant lineage.


1. Gibbs et al Nature 2011
2. Gibbs et al Molecular Cell 2014
3. Abbas et al Current Biology 2015


**A sleigh ride through the SNO: Role of S-nitrosylation in plant immunity**


Gary J. Loake[1]

[1]*Institute of Molecular Plant Sciences, University of Edinburgh*


Changes in redox status are a conspicuous feature of immune responses in a variety of eukaryotes, but the associated signalling mechanisms are not well understood. In plants, attempted microbial infection triggers the rapid synthesis of nitric oxide (NO) and a parallel accumulation of reactive oxygen intermediates (ROIs). In this context, I will discuss our work on S-nitrosylation, the addition of an NO moiety to a protein cysteine thiol to form an S-nitrosothiol, which is emerging as a key regulator of the plant defence response, controlling ROI synthesis, the accumulation of the immune activator, salicylic acid, hypersensitive cell death and defence at the cell wall.

**Regulation of nitric oxide by phytoglobins**


Kim Hebelstrup[1]

[1]*Aarhus University*


Phytoglobins (a.k.a. plant hemoglobins) are present in all known plant genomes. Three classes (1 to 3) of phytoglobins have been identified. Functions of the class 3 type are mostly unknown, whereas the class 1 and 2 phytoglobins have been associated with modulation of nitric oxide (NO) in developmental processes and in stress responses in different plant species. Under normoxic conditions the gene expression pattern of phytogobins is related to cell- and tissue-specific regulation of NO levels in responses to different environments and developmental stages. However, during hypoxic stress conditions class 1 phytoglobin gene expression is highly upregulated in all plant tissues, where phytoglobin plays a role in reducing nitrogen loss through NO emission. NO is a central molecule in the distinct signaling pathways of the responses towards necrotrophic or biotrophic pathogens, and phytoglobin gene overexpression or gene silencing interferes with progression of infections in very different types of plant-pathogen interactions in a pattern related to modulation of NO levels.

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**Ethylene modulation of reactive oxygen species signaling by flavonoid antioxidants in guard cells**

Justin Watkins[1] and Gloria Muday[1]
[1]*Wake Forest University*

This project examined the role of ethylene in modulating signaling in guard cells, which regulate the aperture of stomatal pores on leaf surfaces. The hormone abscisic acid (ABA) triggers stomatal closure through a signaling pathway that generates hydrogen peroxide, a reactive oxygen species (ROS) second messenger. ROS are produced in transient bursts, and their accumulation must be minimized by antioxidants to keep concentrations from reaching damaging levels within the cell. Here we show that ethylene works antagonistically to ABA by inducing flavonol antioxidants in *Arabidopsis* and tomato guard cells. Flavonol accumulation in guard cells, but not surrounding pavement cells, was visualized with confocal imaging of a flavonol-specific dye. Consistent with flavonols in guard cells acting as antioxidants, increased levels of ROS were detected using the ROS sensor DCF in guard cells of *tt4*, an Arabidopsis mutant and *are*, a tomato mutant, which both have defects in flavonol synthesis. ROS levels are conversely decreased in guard cells of *aw,* a tomato mutant that synthesizes elevated levels of flavonols. Guard cells of the *are* and *tt4* mutants were more sensitive to ABA-induced closure than WT, while guard cells of the *aw* mutant are less sensitive. This suggests flavonols may dampen the ABA-dependent ROS burst that drives stomatal opening. Ethylene treatment of wild- type tomato and *Arabidopsis* plants increased flavonol accumulation in guard cells; however, no flavonol increases were observed in *Neverripe* and *ein2-5,* which have defects in ethylene receptors. Consistent with lower levels of ROS due to elevated flavonols, ethylene treatments delayed ABA- mediated stomatal closure. Together these results are consistent with ethylene modulating guard cell signaling by increasing flavonol accumulation and decreasing ROS levels. (Supported by USDA NIFA fellowship #2014-67011-22277).

**4:00-5:35 pm Concurrent 4A: Hormone Signaling**

**Combining imaging and modeling to understand how hormonal signals drive self- organization dynamics at the meristem**

Teva Vernoux[1]
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Plant aerial organs are initiated sequentially at the shoot apex at precise spatial positions, generating spatio-temporal patterns of organogenesis that establish the primary architecture of the shoot, the phyllotaxis. Modeling and wet experiments have shown that auxin-based inhibitory fields are central to the dynamics of organogenesis at the shoot apex, making it a system of choice to address how spatio-temporal changes in signal distribution and signaling capacities regulate developmental patterning in space and time in this self-organizing system. We have recently discovered a second-type of field modulating cytokinin signaling activity. Cytokinin-based inhibitory fields control specifically the timing of organ initiation at the shoot apex and we have further shown that this timing is particularly prone to noise. I will show that a stochastic model of phyllotaxis allows understanding how the timing of organogenesis is influenced by biological noise in phyllotaxis. I will discuss the predictions and consequences of this new stochastic view of phyllotaxis, focusing notably on how it helps understanding the properties of hormone signaling at the shoot apex.

**Cytokinin: Beyond Two Component Signaling**

Christian Burr[1], Carly Sacks[1], Tracy Raines[1], Masha Yamburenko[2], G. Eric Schaller[2], and Joseph Kieber[1]
*[1]Department of Biology, University of North Carolina, [2]Department of Biological Sciences, Dartmouth College*

Cytokinins are $N^6$-substituted adenine derivatives that have been implicated a wide variety of plant growth and development processes. A basic framework for cytokinin signal transduction has emerged that is similar to two-component phosphorelays, which rely on the transfer of phosphates between alternating histidine and aspartic acid residues. Cytokinins are perceived by a family of histidine kinase receptors (AHKs), which, following binding of cytokinin, transfer a phosphoryl group to the histidine phosphotransfer proteins (AHPs), which in turn donate the phosphate to the response regulators proteins (ARRs) thereby regulating their activity. The ARRs fall into two groups, the type-A and type-B ARRs, which act as negative and positive elements in cytokinin signaling respectively. Two-component elements are partially functionally redundant in mediating the response to cytokinin and in various roles in regulating plant growth and development.
We are characterizing the mechanism underlying cytokinin perception and signaling in both Arabidopsis and rice, and are exploring how this two-component signaling pathway modulates the many processes regulated by cytokinin. We have examined the cytokinin- regulated transcriptional network, including extensive characterization of the cytokinin-regulated transcriptome and binding of the type-B ARRs to their genomic targets. We have characterized the role of several of these outputs in cytokinin function, focusing on transcription factors. In the monocot rice, we find that cytokinin has a much more extensive effect on the transcriptome as compared to Arabidopsis. Using CRISPR/Cas9 technology, we are isolating loss-of-function alleles of various two-component elements and are determining the effect of their disruption on rice growth and development.

**COP1 is a negative regulator of seed germination in Strigolactone signaling.**

Shigeo Toh[1, 2], Duncan Holbrook-Smith[1], Yuichiro Tshchiya[1, 2, 3], Toshinori Kinoshita[2, 3] and Peter McCourt[1]
*[1]Cell and Systems Biology, University of Toronto, [2]Division of Biological Science, Graduate School of Science, Nagoya University, [3]Institute of Transformative Bio-Molecules, Nagoya University*

Strigolactones (SLs) are host factors that stimulate seed germination of parasitic plant species *Striga*. To understand the roles of SLs in seed germination, it is necessary to develop a tractable experimental system using model plants such as Arabidopsis. We reported that thermoinhibition, which involves exposing seeds to high temperatures, uncovers a clear role for SLs in promoting Arabidopsis seed germination. Both SL biosynthetic and signaling mutants showed increased sensitivity to seed thermoinhibition. The synthetic strigolactone GR24 does not rescued max2-1 seed germination. Hormone analysis revealed that SLs alleviate thermoinhibition by modulating levels of the two plant hormones, GA and ABA. Recently, we also reported that GR24 directly bind the HTL α/β hydrolase in Arabidopsis in vitro. Strigolactones promoted an interaction between HTL and the F-box protein MAX2 in yeast. We also found *htl* mutant in our GR24 insensitive screening using thermoinhibiton. These results suggest that HTL is involved in SL signaling during seed germination in Arabidopsis. Molecular analysis using a hypocotyl elongation assay showed SLs regulate the nuclear localization of the COP1 ubiquitin ligase, which in part determines the levels of light regulators such as HY5. Genetic analysis revealed that *cop1* single mutant, *cop1 max2* double mutant and *cop1 htl-3* double mutant showed thermo-tolerant seed germination phenotype. These results indicated that COP1 is a negative regulator of seed germination and is genetically at or downstream of MAX2 and HTL in SL signaling with respect to seed germination.

**Nitrate signaling via Abscisic Acid release from inactive conjugates in Arabidopsis root tips.**

Christine Ondzighi-Assoume[1], Sanhita Chakraborty[2] and Jeanne Harris[2]
*[1]University of Tennessee, [2]University of Vermont*

Nitrate is an essential nutrient; as such, plants continuously sense nitrate in the environment, modulating plant growth in response. Root growth is exquisitely sensitive to changes in environmental nitrate, either inhibiting or stimulating growth depending on concentration, location and physiological context. Root branching in response to local nitrate signals had been previously shown to require abscisic acid (ABA) signaling, but the mechanism was unknown. We showed, using a combined immunofluorescence, immunogold and ELISA approach, that after an increase in environmental nitrate, ABA gradually accumulates in the cytoplasm of Arabidopsis root tip cells. We found that nitrate-induced ABA accumulation was preceded by an induction of the *BETA-GLUCOSIDASE 1* (*BG1*) gene, which encodes an enzyme that releases ABA from the inactive ABA glucose ester. Mutants that lack AtBG1 function are unable to stimulate root-tip ABA accumulation in response to a rise in environmental nitrate. We found that ABA strongly induces expression of nitrate-responsive genes, placing ABA signaling squarely within the nitrate signaling pathway in the root, and that the At*bg1* mutant has reduced expression of the nitrate-inducible NITRATE REDUCTASE 1 (*NIA1*) gene under control conditions.
Our immunofluorescence approach revealed that ABA accumulation peaks in the root tip endodermis, cortical/endodermal initial and endodermal daughter cell, with weaker accumulation in the quiescent center. This pattern of accumulation mirrors the expression pattern of SCARECROW (SCR), a transcription factor involved in radial patterning of the root tip that regulates endodermal and quiescent center fate. SCR represses ABA signaling by inhibiting expression of the transcription factors, ABI4 and ABI5. We found that both ABA and nitrate reduce expression of an *SCR:erGFP* reporter gene, suggesting that ABA and nitrate may stimulate ABA-regulated gene expression in the root, by inhibiting an inhibitor, SCR.
We are currently extending this approach to examine ABA localization in the roots of other species, with the intent of using this tool for comparative physiology of different plant taxa.

**4:00-5:30 pm Concurrent 4B: Biotic Interactions**

**Vascular hijack by parasitic plants**

Ken Shirasu[1]
*[1]RIKEN Center for Sustainable Resource Science*

Parasitic plants retrieve different molecules from host plants, which can result in severe growth penalties for the host. This is of particular interest, when parasitic plants of the Orobanchaceae family infect crops such as maize, sorghum (*Striga* sp.) or sunflowers (*Orobanche sp.)*.
Molecular studies on these obligate parasites are hampered by the lack of genetic resources and efficient transformation protocols. We therefore established *Phtheirospermum japonicum* (Pj), a hemi-parasitic Orobanchaceae species, native to East Asia, as model plant to study parasitic plant-host interactions. Like many other hemi-parasitic plants, Pj has a relatively wide host-range, which enabled us to use *Arabidopsis thaliana* as host species. Pj taps into the host vasculature by a so called xylem bridge. The xylem bridge is imbedded in a specialized feeding organ of the parasite, the haustorium. While nutrient fluxes to parasitic Orobanchaceae were studied previously, there is only very limited information on transfer of parasite-derived molecules to host plants.
We used fluorescent dyes to visualize long-distance transport from host to parasite across the haustorium. Rapid accumulation of fluorescent dye in the haustorium suggests high conductivity of haustoria. We further observed that host plants reciprocally also received signalling molecules from the parasite. These molecules are well studied plant hormones and entered the vascular system of the host to be then transported towards the host shoot apex. Arabidopsis mutants were used to study the source of these hormones and to characterize

their effect. Our experiments provide evidence that these hormones are actively perceived by the host and provoke enhance secondary growth of host roots above haustoria.
In conclusion, we show that vascular transport between parasitic plants and host plants can be studied at the molecular level using the parasitic plant *Phtheirospermum japonicum* and *Arabidopsis thaliana.* Transport across haustoria is bidirectional and actively reshapes root morphology and size of parasitized plants.

**Pipecolic acid – a central regulator of plant systemic acquired resistance and defense priming**

Jürgen Zeier[1]
[1]*Department of Biology, Heinrich Heine University*

Systemic acquired resistance (SAR) is an inducible plant immune response that is initiated by a localized inoculation of leaves with virulent or avirulent phytopathogens. Plants with activated SAR exhibit enhanced resistance in the entire foliage towards infections by many biotrophic and hemibiotrophic pathogens, and are primed to more effectively activate defense responses to future microbial attack. SAR induced in *Arabidopsis thaliana* by the bacterial pathogen *Pseudomonas syringae* is accompanied with a strong transcriptional response systemically in the foliage that includes up-regulation of genes involved in multiple stages of defense signaling, enhanced expression of pathogenesis-related (PR) genes, and downregulation of photosynthetic and growth- related genes. In the course of SAR activation, the lysine-derived non-protein amino acid pipecolic acid (Pip) strongly accumulates in both *P. syringae*-inoculated and in non-inoculated distal leaves. Pip is generated in dependence of AGD2-LIKE DEFENSE RESPONSE PROTEIN1 (ALD1), an aminotransferase gene which is systemically up-regulated in the plant upon *P. syringae* inoculation. Pip has a widespread occurrence in Angiosperms and is generated to high levels after bacterial, fungal, or viral infection in many different plant species, including rice, potato, tobacco, soybean, and Arabidopsis. Notably, the systemic accumulation of Pip in the foliage is necessary for SAR establishment and the associated systemic elevations of the phenolic defense hormone salicylic acid (SA) (Návarová et al., 2012). Pip exerts its SAR-inducing capacity in dependence of FLAVIN-DEPENDENT-MONOOXYGENASE1 (FMO1). A second critical SAR-regulatory metabolite is SA, which is synthesized in plastids by ISOCHORISMATE SYNTHASE1 (ICS1) from chorismate. Recent findings from our laboratory indicate that Pip regulates SAR via a major, SA-dependent and a minor, SA- independent activation pathway (Bernsdorff et al., 2016). This is illustrated by the full absence of the normally observed transcriptional SAR response in *ald1* and *fmo1* knockout plants and a strongly diminished but not fully abrogated response in *ics1* plants after *P. syingae* inoculation. The two central SAR regulatory metabolites Pip and SA act synergistically in the induction of *PR* gene expression but also trigger separate signaling pathways that can function independently from each other. Moreover, Pip orchestrates SA-dependent and SA-independent priming of pathogen responses in a FMO1-dependent manner. Therefore, a Pip/FMO1-signaling module acts as an indispensable switch for the activation of SAR and associated defense priming events, and SA amplifies Pip- triggered responses to different degrees in the distal tissue of SAR-activated plants. The talk will highlight the interplay between the two critical signals Pip and SA in SAR and defense priming, involve novel aspects of pipecolic acid metabolism, and introduce a novel regulatory component downstream of Pip and SA in SAR activation.

Literature:
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**The clubroot pathogen *Plasmodiophora brassicae* controls plant hormone homeostasis by degradation, conjugation and methylation to alter plant defense responses**

Jutta Ludwig-Müller[1] and Sabine Jülke[1]
[1]*Technische Universität Dresden*

The clubroot disease of Brassicaceae results in the formation of large root galls induced by the obligate biotrophic protist *Plasmodiophora brassicae*. The recently published genome sequence of this unique organism helped to shed light on mechanisms how the protist controls the hormone homeostasis of the host plants. The growth promoting hormones auxin and cytokinin have been linked to gall size. Indole-3-acetic acid (IAA) is conjugated to various amino acids by a protein from the GH3 family of auxin conjugate synthetases identified in *P. brassicae* (PbGH3) and thereby reversibly inactivated. The genome of the protist also encodes, next to two genes for isopentenyltransferases, a gene for a cytokinin oxidase. The respective enzyme is able to degrade various cytokinins *in vitro*. It was hypothesized, based on previous microarray datasets, that the defense responses of the host plant are altered by the clubroot pathogen. We found that the PbGH3 protein is also able to conjugate jasmonic acid (JA) to various amino acids. However, the activity with isoleucine is very low, indicating that the active JA-Ile conjugate that is recognized in the host *Arabidopis thaliana* for defense induction is formed only to a small extent. Formation of other JA conjugates by the pathogen might prevent the formation of the active conjugate by the host. Finally, salicylic acid (SA) is methylated by a specific methyl transferase of *P. brassicae* that includes a secretion signal. This indicates that the enzyme might be active in the host cell and not in *P. brassicae*. We showed that Me-SA is the major transport form in clubroot infected *Arabidopsis* plants from roots to inflorescences. These biochemical findings on the manipulation of the host plant's hormone homeostasis by *P. brassicae* will be discussed in a biological model.

**DELLA regulates arbuscular mycorrhiza formation by interacting with the central symbiosis transcription factor CYCLOPS**

Priya Pimprikar[1], Samy Carbonnel[1], Michael Paries[1], Katja Katzer[1], Verena Klingl[1], Monica Bohmer[1], Leonhard Karl[1], Martin Parniske[1], and Caroline Gutjahr[1]
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Arbuscular mycorrhiza (AM) symbiosis with glomeromycotan fungi is a widespread strategy of plants to acquire mineral nutrients from soil. Root colonization by AM fungi culminates in the formation of highly branched structures called arbuscules that release the mineral nutrients to the plant. Arbuscule formation requires DELLA proteins and is accordingly inhibited by gibberellin. However, it remained unknown how DELLA promotes arbuscule formation mechanistically. During colonization perception of fungal signals triggers nuclear calcium spiking, which is decoded by a nuclear localized calcium and calmodulin dependent kinase (CCaMK). CCaMK interacts with and phosphorylates the transcription factor CYCLOPS that activates downstream AM signaling. We found that DELLA physically interacts with CYCLOPS to regulate target genes specifically involved in arbuscule formation such as *RAM1*, which is required for arbuscule branching. CYCLOPS directly binds to a *cis*-element in the *RAM1* promoter and likely recruits DELLA to the DNA. Ectopic expression of degradation resistant DELLA-delta17 induces *RAM1* and other genes involved in arbuscule formation in the absence of the fungus. Furthermore, ectopic *RAM1* expression supports arbuscule formation in a *cyclops* mutant and in presence of GA placing *RAM1* downstream of the CYCLOPS-DELLA complex. We reveal a transcription factor complex, which integrates symbiosis and GA signaling possibly to adjust symbiosis development with the plant physiological state.

**Protein Phosphatase 2A as a post-translational regulator of salicylic acid dependent pathogenesis responses**

Moona Rahikainen[1], Andrea Trotta[1], Guido Durian[1], Sara Alegre[1], Barbara Moffatt[2], Stéphane Ravanel[3], Erich Glawischnig[4] and Saijaliisa Kangasjärvi[1]

*[1]Department of Biochemistry, Molecular Plant Biology, University of Turku, [2]Department of Biology, 200 University Avenue, University of Waterloo, [3]Laboratoire de Physiologie Cellulaire & Végétale, CNRS UMR5168, INRA UMR1417, CEA, Université Grenoble Alpes, [4]Department of Plant Sciences, Technische Universität München*

Plant immunity is governed by converging signaling pathways, which are largely regulated through reversible protein phosphorylation, light-dependent formation of reactive oxygen species (ROS), and associated hormonal signalling. We have addressed the role, regulation and interactions of protein phosphatase 2A (PP2A) in plant immunity. A combination of genetic, proteomic and metabolomic analysis revealed that PP2A regulatory subunit B'*y* (PP2A-B'*y*) is required to suppress salicylic acid mediated pathogenesis responses and metabolic signatures triggered by photorespiratory ROS signals in Arabidopsis. Mutant analysis, together with visualization of protein interactions revealed that PP2A-B'*y* mediates post-translational regulation of methionine metabolism and modulates the formation of methylated indole glucosinolates in Arabidopsis leaves. Moreover, PP2A-B'*y* physically interacts with and negatively regulates a CALCIUM-DEPENDENT PROTEIN KINASE (CPK), a key mediator of salicylic acid dependent immune responses. The multifaceted function of PP2A in signalling and responding to biotic stress agents in plants will be presented.

**4:00-5:30 pm Concurrent 4C: Reproductive Development**

**The contribution of the 'antiflorigen' TFL1 to inflorescencearchitecture**

Cheol Woong Jeong[1], Nobutoshi Yamaguchi[2], Koji Goto[3] and Doris Wagner[1]

*[1]Department of Biology, University of Pennsylvania, [2]Nara Institute of Science and Technology, [3]Research Institute for Biological Sciences, Okayama Prefecture*

Like its close homolog FLOWERING LOCUS T (FT), TERMINAL FLOWER 1 (TFL1) is a small mobile protein. However unlike FT, which is an activator of flowering, TFL1 negatively inpacts the switch to flower formation. Moreover, recent data has suggested that while FT acts predominantly as a co-activator, TFL1 likely acts as a co-repressor (Hanano S., Goto K. 2011). Confirmation of this hypothesis awaits identification of immediate early target genes of TFL1. Here we provide evidence that TFL1 executes its critical role in control of inflorescence architecture in large part via repression of the floral fate promoting *LEAFY* gene. TFL1, like FT, is thought to be recruited to its target loci via the bZIP transcription factor FD. On the basis of chromatin immunoprecipitation (ChIP), TFL1 and FD bind to evolutionarily conserved bZIP binding sites at the *LFY* locus and TFL1 association with this region is reduced in the *fd* mutant. A *LFY* reporter that does not contain the bZIP binding sites displays ectopic expression. Finally, inducible activation of TFL1 led to decreased *LFY* accumulation. Our data support the idea that TFL1 represses its immediate early target genes. It also fits well with prior genetic studies, which had revealed that *tfl1* loss-of-function mutants phenocopy *LFY* gain-of-function mutants and vice versa. Moreover, *TFL1* and *LFY* expression is largely non-overlapping. Further studies are aimed at precise placement of TFL1 in the regulatory network that controls proper timing of flower formation.

categories: Development/reproductive and flowering

References:

Hanano S. and Goto K. (2011) *Arabidopsis* TERMINAL FLOWER1 Is Involved in the Regulation of Flowering Time and Inflorescence Development through Transcriptional Repression Plant Cell 23 (9) 3172-3184.

**Flowering and plant architecture in the perennial model *Arabis alpine***

Maria Albani[1]
[1]*University of Cologne*

Perennials maintain vegetative growth after flowering showing a complex shoot architecture consisting of flowering branches, vegetative branches or dormant buds. The topology and fate of meristems differ between species but in general flowering and vegetative branches or dormant buds are organized in recurrent distinct zones that appear in a stable pattern within a species. We use the Brassicaceae species *Arabis alpina* as a model to study flowering and vegetative traits in perennials. Different behaviors of axillary meristems have been described in *A. alpina*: I) axillary meristems that develop into axillary branches that commit to reproductive development, II) dormant axillary meristems and III) axillary meristems that develop into vegetative branches after floral transition. This growth pattern is a result of coordinated action of floral repressors regulating maintenance of vegetative growth after flowering. Previous studies in *A. alpina* demonstrated that the MADS box transcription factor *PERPETUAL FLOWERING 1* (*PEP1*) regulates maintenance of vegetative growth after flowering. We are interested in understanding the downstream effects of maintenance of vegetative growth mediated by the *Type III* branches. Auxin measurements indicate that return to warm temperatures after vernalisation results in transient increase in auxin levels in different stem parts which correlates with accelerated bud outgrowth.

**A non-canonical auxin-sensing mechanism is required for organ morphogenesis in Arabidopsis**

Sara Simonini[1]
[1]*Department of Crop Genetics, John Innes Centre, Norwich Research Park*

Tissue patterning in multicellular organisms is the output of precise spatio-temporal regulation of gene expression coupled with changes in hormone dynamics. In plants, the hormone auxin regulates growth and development at every stage of a plant's life cycle. Auxin signaling occurs through binding of the auxin molecule to a TIR1/AFB F-box protein allowing interaction with Aux/IAA transcriptional repressor proteins. These are subsequently degraded via the 26S proteasome leading to de-repression of auxin response factors (ARFs). How auxin is able to elicit such a diverse range of developmental responses through a single signaling module has not yet been resolved. Here we present an alternative auxin-sensing mechanism in which the auxin response factor ARF3/ETTIN through interactions with process-specific transcription factors controls expression of downstream targets. This non-canonical hormone-perception mechanism is important for coordinating growth and patterning in diverse developmental contexts such as gynoecium morphogenesis, lateral root emergence, ovule development and secondary branch formation. Disrupting the auxin-sensing ability induces morphological aberrations with consequences for plant fitness. Therefore, our findings introduce a novel transcription factor-based mechanism of hormone perception in plants.

**Gibberellins are essential for cucumber female flower development**

Maria João Pimenta Lange[1] and Theo Lange[1]
[1]*Braunschweig University of Technology, Institute of Plant Biology, Germany*

Gibberellins (GAs) are hormones that play a central role for plant growth and development. The contribution of GAs for male and bisexual flower development is well studied. The stamen is the main source of bioactive GAs necessary for the development of the floral organs. However, little is known about the contribution of GAs for female flower development. Detailed analysis of endogenous GAs and transcript levels of GA signalling genes revealed that GAs are highly regulated during cucumber female flower development. But, unlike male flowers, our results suggest that cucumber female flowers produce mainly the biological inactive precursor $GA_9$ in ovaries that moves to petals and sepals where it is further converted to bioactive $GA_4$. To verify our hypothesis,

deuterated GAs were injected into ovaries. Deuterated $GA_9$ is translocated from ovaries to petal/sepal tissues. To confirm the importance of ovary derived $GA_9$ for flower development, a catabolic GA 2-oxidase from pumpkin (CmGA2ox1) was expressed transiently in cucumber ovaries. This approach reduced the levels of precursor $GA_9$ and bioactive $GA_4$ significantly and flower development was arrested completely but could be restored by application of deuterated $GA_9$ to the petals. Altogether, our results suggest that ovary derived $GA_9$ is sufficient for cucumber female flower development. Since bioactive GAs can promote sex reversion of female flowers is now tempting to speculate that movement of a biological inactive precursor, instead of the hormone itself, might help female sex maintenance.

**IQ-domain proteins connect auxin and calcium signaling during Arabidopsis development**

Jos R. Wendrich[1], Pieter Mijnhout[1], Bert De Rybel[2] and Dolf Weijers1
*[1]Wageningen University, [2]Wageningen University and VIB Ghent University*

Plant development follows a highly coordinated route and many of its processes are controlled by the phytohormone auxin. Embryonic root apical meristem initiation is a key developmental event where auxin plays an important role, mainly through the action of the AUXIN RESPONSE FACTOR5/ MONOPTEROS (MP) transcription factor. Disruption of MP function leads to a variety of defects on different levels, ranging from transcriptional responses to disturbed cellular processes. Although a number of downstream targets of MONOPTEROS have been identified and characterized, an open question remains how cellular processes that govern cell shape and function are directed by this transcription factor. By comparing different transcriptomic datasets we have identified a subclade of IQ-domain proteins acting downstream of MP. Both functional and molecular characterization show that IQD15-18 are transcriptionally controlled by auxin, that they interact with Calmodulins and microtubules *in vivo*, and that subcellular localization of IQD18 protein is cell cycle-dependent. Loss- and gain- of-function analyses revealed a role for these proteins in both auxin and calcium signaling. These findings place the IQD15-18 proteins at the hinge between two important signaling pathways and shed light on how cellular processes may be directed by MONOPTEROS.

**Manipulating gibberellin signalling in developing wheat grain for improved yield and quality**

Aakriti Wanchoo-Kohli[1], Simon Vaughan[1], Michael Holdsworth[2], Andy Phillips[1] and Peter Hedden[1]
*[1] Plant Biology and Crop Sciences, Rothamsted Research, [2] Division of Plant and Crop Sciences, The University of Nottingham*

During germination wheat embryos produce gibberellin (GA) which induces the production of α-amylase by the aleurone layer that causes the subsequent hydrolysis of starch in the endosperm. Under certain environmental conditions GA can cause the premature induction of α-amylase resulting in degraded starch in the mature grain and poor quality flour. However, while GA is proposed to have a negative effect on flour quality, it is also essential for early grain development. As these effects are separated both temporarily and spatially in the grain, it may be possible to improve both grain yield and flour quality by manipulating GA signalling in specific tissues at specific times. We hypothesise that increasing GA content in early stages of development may promote grain size without having a negative impact on flour quality, while reducing GA content late in development, or conferring insensitivity to GA in specific tissues may improve flour quality, without affecting grain size. In order to test this and to obtain a better understanding of the role of GA in grain development, constructs were designed to alter GA metabolism or signalling in the seed-coat, endosperm, embryo or aleurone of developing wheat grains. The tissue and temporal specificity of each promoter was confirmed by co-transformation with GFP reporter constructs. To identify homozygous plants a reliable Q-PCR method using TaqMan assays was developed and zygosity determined in the T2 generation for each construct. Grain yield and quality in the transgenic lines were compared with azygous segregants in terms of grain number, weight, size and shape; α-amylase activity, protein content, grain hardness and moisture. Transcript levels of the transgenes were also

measured using qRT-PCR to determine linkages between genotype and phenotype.

**Friday, June 24, 2016**

**9:00-10:30 am Plenary V: Hormones & Development**

**TRANSPORTER OF IBA1 links auxin and cytokinin to regulate root architecture**
Lucia Strader[1]
[1]*Washington University in St. Louis*

Root system architecture and consequently lateral root formation and are critical for soil exploration by plant roots, allowing for uptake of water and nutrients. Conversion of the auxin precursor indole-3-butyric acid (IBA) to active auxin (indole-3-acetic acid; IAA) modulates lateral root formation. However, mechanisms governing IBA-to-IAA conversion have yet to be elucidated. We identified TRANSPORTER OF IBA1 (TOB1) as a vacuolar IBA transporter that limits lateral root formation, likely by sequestering IBA in the vacuole to prevent its contribution to the active auxin pool that drives lateral root formation. Moreover, *TOB1* transcripts and protein accumulate in response to the phytohormone cytokinin, which inhibits lateral root formation. The increased production of lateral roots in *tob1* mutants, TOB1 transport of IBA into the vacuole, and cytokinin- regulated *TOB1* expression suggest a mechanism linking cytokinin signaling and IBA contributions to the auxin pool to ultimately modulate root system architecture.

**11:00-12:35 pm Concurrent 5A: Vegetative Development**

**LLM-domain B-GATAs control stomata formation downstream from light and PIF transcription factors**
Carina Klermund[1], Quirin Ranftl[1], Emmanouil Bastakis[1], Julia Diener[1] and Claus Schwechheimer[1]
[1]*Technical University of Munich, Freising, Germany*

LLM-domain B-GATAs are a subfamily of the thirty-membered GATA transcription factor family from *Arabidopsis thaliana*. We have shown that all six Arabidopsis LLM- domain B-GATAs have redundant functions in the control of germination, greening, phyllotaxy, accesaroy meristem formation, flowering time, and senescence downstream from auxin, cytokinin, gibberellin, and light signaling.
Here, we show that mutants of *Arabidopsis thaliana* LLM-domain B-GATA genes are defective in stomata formation in hypocotyls. Conversely, stomata formation is strongly promoted by overexpression of various LLM-domain B-class GATA genes, most strikingly in hypocotyls but also in cotyledons. Genetic analyses indicate that these B-GATAs act upstream from the stomata formation regulators *SPCH (*SPEECHLESS), *MUTE*, and *SCREAM/SCREAM2* and downstream or independent from the patterning regulators *TMM* (*TOO MANY MOUTHS*) and *SDD1* (*STOMATAL DENSITY AND DISTRIBUTION1*). The effects of the GATAs on stomata formation are light-dependent but can be induced in dark-grown seedlings by red, far-red or blue light treatments. *PHYTOCHROME INTERACTING FACTORS* (*PIFs*) mutants form stomata in the dark and, in this genetic background, also *GATA* expression is sufficient to induce stomata formation in the dark. Since the expression of the LLM-domain B-GATAs *GNC* and *GNL* as well as that of *SPCH* is red light-induced but the induction of *SPCH* is compromised in a *GATA* gene mutant background, we hypothesize that PIF- and light-regulated stomata formation in hypocotyls is critically dependent on LLM-domain B-GATA genes.

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**Identifying Gibberellic-Acid transport mechanisms in *Arabidopsis***

Eilon Shani[1]
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Gibberellins (GAs) are plant hormones that promote a wide range of processes in plants and are commonly used in agriculture. While GA signaling is well understood, little is known about the process of GA transport or the regulation of GA distribution in the plant. We have utilized a unique bioactive fluorescently-labeled GAs (GA-Fl) to screen for *Arabidopsis* mutants deficient in GA transport. We identified the transporter NPF3 and show that it efficiently transports GA across cell membranes both *in vitro* and *in vivo*. Expression of *NPF3* occurs in the root endodermis and is repressed by GA. NPF3 is targeted to the plasma membrane and subject to rapid BFA-dependent recycling. We show that abscisic acid (ABA), an antagonist of GA, is also transported by NPF3 in vitro. ABA promotes *NPF3* expression and GA-Fl uptake in plants. On the basis of these results, we propose that GA distribution and activity in *Arabidopsis* is partly regulated by NPF3 acting as an influx carrier and that GA–ABA interaction may occur at the level of transport.

**A role for auxin methylation during plant development**

Mohamad Abbas[1,4], Jorge Hernández-García[1], Stephan Pollmann[2], Matías Zurbriggen[3], Jiri Friml[4], Miguel A. Blázquez[1], David Alabadí[1]
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Auxin gradients are instrumental for the differential growth that causes organ bending upon tropic stimuli and curvatures during plant development. Local differences in auxin concentrations are achieved by uneven distribution of auxin transporters, but it is not clear if other mechanisms, involving auxin homeostasis, are also a relevant regulatory target for the formation of auxin gradients. We have found that auxin methylation is absolutely required for correct auxin distribution across the hypocotyl, in particular during the response to gravity. We have found that loss-of-function mutants in Arabidopsis *IAA CARBOXYL METHYLTRANSFERASE1* (*IAMT1*) prematurely open the apical hook and their hypocotyls are impaired in gravitropic reorientation. This defect is linked to an increased polar auxin transport in the *iamt1* mutant, which causes the accumulation of auxin on either side of the gravistimulated hypocotyl. Partial inhibition of polar auxin transport in the *iamt1* mutant resulted in the restoration of normal gravitropic reorientation. We propose that IAA methylation is necessary to restrict polar auxin transport within the range of auxin levels that allow differential responses.

**A Model Integrating Cytokinin into Regulation of Shoot Branching by Light Signals**

Tesfamichael H Kebrom[1] and John E Mullet[1]
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Shoot branching is regulated by environmental and hormonal signals. The proportion of red (R) and far red (FR) lights (R:FR ratio) from the sun incident on leaves is one of the environmental signals that regulate shoot branching. Red light is absorbed by leaves for photosynthesis whereas FR is reflected or transmitted. The R:FR ratio is perceived by the photoreceptor phytochrome B (phyB). High R:FR activates phyB that promotes bud outgrowth by modulating the expression of numerous genes including repressing the expression of the bud dormancy inducing *teosinte branched1* (*tb1*) gene. The microenvironment of plants grown in dense canopies is enriched with shade signals of FR light reflected by leaves, and thus the R:FR ratio is lower and phyB signaling is less active. The expression of *tb1* is elevated when phyB is inactivated by low R:FR or in phyB null mutants and buds become dormant soon after they are formed. Among the plant hormones that regulate shoot branching,

auxin and strigolactones synthesized in the shoot apex and roots, respectively, induce bud dormancy without entering into buds whereas cytokinins synthesized in roots and stem promote bud outgrowth by acting locally within the bud. The inhibition of bud outgrowth by phyB deficiency in the phyB null mutant (*phyB-1*) sorghum has been linked to the expression of genes that could reduce cytokinin levels in the bud (Kebrom and Mullet, 2016). We propose a model integrating cytokinin in the regulation of shoot branching by light signals perceived by phyB.

**Carbon availability controls shoot growth through sugar-induced cytokinin biosynthesis and transport in *Arabidopsis***

Takatoshi Kiba[1], Mikiko Kojima[1] and Hitoshi Sakakibara[1]
[1]*RIKEN CSRS*

Carbon nutrient availability is a major regulatory factor of plant growth and development. Although a plant hormone cytokinin, which plays an important role in various aspects of plant growth and development, has been implicated in the carbon availability-dependent regulation, the details of their involvement remain to be elucidated. Here we report that shoot growth enhancement under elevated $CO_2$ is mediated by sugar-induced cytokinin biosynthesis and transport in *Arabidopsis thaliana*. Treatment of Arabidopsis seedlings in elevated $CO_2$ resulted in an accumulation of cytokinin precursors in shoots and roots that preceded shoot growth enhancement, indicating that cytokinin *de novo* biosynthesis is activated. Among genes involved in the *de novo* biosynthesis, only two genes, an adenosine phosphate-isopentenyltransferase gene, *AtIPT3*, and a cytochrome P450 monooxygenase gene, *CYP735A2,* were consistently induced in response to elevated $CO_2$ in the root. In addition, *ABCG14*, a gene involved in root-to-shoot transport of cytokinin, were induced similarly to *AtIPT3* and *CYP735A2*. The expression of these genes was inhibited by dark treatment and a photosynthesis inhibitor, DCMU, under elevated $CO_2$ and was enhanced by sugar supplement, indicating that photosynthetically generated sugars are responsible for the induction. The *ipt3 ipt5 ipt7* and *cyp735a1 cyp735a2* mutants, defective in elevated $CO_2$-induced cytokinin accumulation, were impaired in shoot growth enhancement under elevated $CO_2$, demonstrating the requirement of cytokinin *de novo* biosynthesis in the growth response. We propose that plants employ an intricate system to regulate shoot growth in response to elevated $CO_2$, in which photosynthetically generated sugars induce cytokinin *de novo* biosynthesis and transport in the root for shoot growth regulation.

**ABA is a modulator of endodormancy release in grapevine buds**

E Or[1], C Zheng, and T Halaly[1]
[1]*Department of fruit tree sciences, Volcani center, ARO, Israel*

In warm-winter regions, bud dormancy release poses a major obstacle to commercial viticulture. Artificial substitutes for chilling are thus mandatory. Induction of respiratory stress by artificial stimuli s(uch as hydrogen cyanamide, HC) leads to dormancy release of endodormant buds via an uncharacterized biochemical cascade of events, which we try to dissect in order to design proper alternatives. We formerly proposed that (1) ABA inhibits bud dormancy release via repression of meristem activity; (2) the enhancing effect of dormancy release stimuli involves reduction in ABA level and/or changes in response to ABA. Our data show that ABA indeed inhibits dormancy release in grapevine buds and attenuates the advancing effect of HC. However, HC-dependent recovery was detected, and was affected by dormancy status. Regulation of central players in ABA metabolism (VvNCED and VvABA8'OH) correlated with decreased ABA and increased ABA-catabolite levels in HC-treated buds. The activity of the major bud VvNCED and VvABA8'OH was verified in vivo, and as predicted, transgenic vines over expressing VvABA8'OH presents enhanced bud break and had an interesting effect on apical dominance. Expression profiling during the natural dormancy cycle revealed that at maximal dormancy, VvNCED1 expression is down regulated while that of starts to drop while levels of VvABA8'OH transcript and ABA catabolites increase sharply. This may provide

initial support for the involvement of ABA metabolism in the execution of natural dormancy as well. We will additionally report on 1) behavior of transgenes with modified ABA response; 2) potential interaction between ABA and other growth regulators (GA, JA, Ethylene); 3) effect of ABA and other stimuli on the bud transcriptom.

**11:00-12:30 pm Concurrent 5B: Novel Methods**

**Imaging phytohormones during development and environmental responses using FRET biosensors**

Annalisa Rizza[1], Wolf B. Frommer[2], and Alexander M Jones[1]
*[1]Sainsbury Laboratory, Cambridge University, [2]Carnegie Institution for Science Department of Plant Biology*

Plants use phytohormones - a suite of mobile small molecules – as potent regulators that coordinate and adjust development to match their environmental conditions. For example, accumulation of gibberellins (GA) is integral to numerous plant growth processes such as germination, tissue enlargement, and fruit set as well as key developmental transitions such as photomorphogenesis and flowering. It is no surprise, then, that accumulation of GA is tightly regulated in a cell-type and temporally specific manner by a series of biosynthetic, modification, catabolic and transport proteins. However, we currently lack methods for high spatiotemporal resolution measurement of GA in living tissues, and this limitation hampers analysis of cell-specific GA accumulations. Using an accelerated biosensor engineering platform, we have developed genetically- encoded, ratiometric fluorescent biosensors for the high-resolution measurement of GA in living tissues. Fluorescence imaging of the Gibberellin Perception Sensor (GPS) in the nuclei of *Arabidopsis* hypocotyls undergoing photomorphogenesis allows comparison of the timing of GA accumulation vs cell expansion. In addition to tracking endogenous GA accumulations, treatment of GPS plants with exogenous GA reveals that GA accumulation is patterned across tissues and varies with the type of GA applied. Potential mechanisms governing these cellular GA patterns and dynamics will be discussed. In the future, GPS can be used to address fundamental questions regarding how multiple signals integrate to control the hormone patterns and dynamics that, in turn, influence plant developmental and environmental responses.

**Comparison of cytokinin metabolism kinetics of two distinct Arabidopsis ecotypes through experimental and computational techniques**

Petr Hosek[1], Klara Hoyerova[1], Petre Dobrev[1], Kiran N. S.[2], Eva Zizkova[1], and Motyka V.[1]
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Until now, almost 70 cytokinin derivatives have been described in higher plants, suggesting how complex the network of interconversions determining the concentration of particular cytokinin forms have to be. Because of such complexity we have previously used mathematical methods for interactive visualisation and analysis of experimental data obtained after exogenous application of four major cytokinins: trans-zeatin, cis-zeatin, dihydrozeatin, and isopentenyladenin in two-hour time span on *Arabidopsis thaliana* Col-0. The metabolic system was divided into four subsystems according to particular incubations and independent multicompartment mathematical models of these subsystems were then constructed. Multiple Monte Carlo optimization of the models was carried out providing estimates of kinetic parameters of major reactions. Subsequent sensitivity analysis and statistical analysis provided further insight into parameter importance and reliability of the estimates. To reveal whether the metabolic machinery determining the concentration of particular cytokinin forms in A.t.
Col-0 is universal for the whole specie or whether the network of reactions is rather specific and might be a subject to adaptational changes, we have analysed distinct *Arabidopsis thaliana* ecotype Van-0 in order to obtain a comparison of kinetic parameter estimates of the major reactions of cytokinin metabolism between Col-0 and Van-0.

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**Selective degradation of Aux/IAA proteins modulates plant development**

Thomas Vain[1], Noel Ferro[2], Deepak Kumar Barange[1], Qian Ma[1], Mattias Thelander[3], Barbora Pařízková[4], Ondřej Novák[4], Siamsa M. Doyle[1], Alexandre Ismail[5], Per Anders Enquist[6], Adeline Rigal[1], Yi Zhang[7], Malgorzata Langowska[1], Karin Ljung[1], Judy Callis[8], Fredrik Almqvist[9], Mark Estelle[7], Laurens Pauwels[10] and Stéphanie Robert[1]
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Auxin phytohormones control most aspects of plant development through a complex and interconnected signaling network. In the presence of auxin, AUXIN/INDOLE-3-ACETIC ACID (Aux/IAA) transcriptional repressors are targeted for degradation by the SKP1- CULLIN1-F-BOX (SCF) ubiquitin-protein ligases containing TRANSPORT INHIBITOR RESISTANT 1/AUXIN SIGNALING F-BOX (TIR1/AFB). Here, we report four small molecules named DEVELOPMENTAL REGULATORS (DRs) requiring AXR1 and $SCF^{TIR1/AFB}$ to modulate plant development. Three DR molecules trigger selective auxin responses at transcriptional, biochemical and morphological levels which are explained by their ability to promote the interaction between TIR1 and a specific subset of Aux/IAA proteins. These results demonstrate the potential of selective auxin agonists to reprogram plant development through a selective degradation of the Aux/IAA transcriptional repressors.

**A novel targeted metabolomic approach in plant hormone analysis**

Jan Šimura[1,2], Ioanna Antoniadi[3], Danuše Tarkowská[1], Jitka Široká[1], Karin Ljung[3], and Ondřej Novák[1,3]
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Plant hormones, otherwise known as phytohormones, are highly bioactive compounds which are directly responsible for organized plant growth and development including flowering, seed germination, senescence and various stress responses. Occurrence and levels of these compounds strongly depend on plant organ, plant age, developmental stage and environmental conditions, they are often present only in minute concentrations and thus their direct quantification provides difficult analytical task.
Phytohormones as a group of diverse compounds could be divided into several structurally different families. In this study we present a new ultra-high liquid chromatography tandem mass spectrometry (UHPLC-MS/MS) targeted method based on complex extraction and selective SPE purification for profiling of active compounds from cytokinin, auxin, brassinosteroid, gibberellin, jasmonate, abscisic acid and salicylic acid families, including their metabolites and precursors (more than 100 analytes). Their biosynthetic and signaling pathways are very complex, often interacting among themselves thus the regulation of various biological processes in plants is controlled by multiple phytohormones. We believe this generalized analytical screening method to be very useful for preliminary phytohormonal screening and studies dealing with phytohormonal crosstalks.

**TISSUE-SPECIFITY OF ABA BIOSYNTHESIS IN RELATION TO ITS ROLES DURING ARABIDOPSIS SEED DEVELOPMENT AND GERMINATION**

Annie Marion-Poll[1], Anne Frey[1], François Perreau[1], Camille Roux[1], Marlène Bailly[1], Boris Collet[1], Gwendal Cueff[1], Gilles Clément[1], Helen North[1], and Loïc Rajjou[1]
*[1]Institut Jean-Pierre Bourgin, UMR1318, INRA, AgroParisTech, CNRS, Université Paris- Saclay*

Abscisic acid (ABA) is a key element in seed development and germination as well as adaptive responses to environmental stresses. The tissue-specific modulation of its endogenous levels by fine-tuning of synthesis and catabolism determines its physiological action. In seeds, ABA is synthesized in genetically distinct tissues, the testa is constituted of maternal tissues derived from ovule integuments, whereas the triploid endosperm and the diploid embryo result from two separate nuclear fusions, called double fertilization.
ABA is derived from carotenoid precursors. To date, genes encoding enzymes responsible for most steps of the ABA biosynthesis pathway have been identified in Arabidopsis. Our group has studied the regulation of genes involved in the conversion and cleavage of carotenoids, specifically zeaxanthin epoxidase, neoxanthin synthase and 9–*cis* epoxycarotenoid dioxygenase (*NCED2*, *3*, *5*, *6* and *9*). NCED enzymes cleave the *cis*-isomers of violaxanthin and neoxanthin to a C15 product, xanthoxin, which is then converted into ABA by two successive enzyme reactions. In contrast to other enzymes of the ABA pathway, the cleavage enzyme is encoded by a multigene family, which potentially allows the precise regulation of xanthoxin production. We have characterized multiple *nced* mutants whose ABA deficiency is restricted to seeds and gained new insights into ABA origin. Analysis of hormone levels showed that *NCED6* specific expression in the endosperm is the major source of ABA during seed development, in contrast to the minor amount produced by other NCEDs expressed in the embryo or mother plant. “Omics” approaches were performed on developing seeds of *nced2 nced5 nced6 nced9* (*qnced*), severely ABA deficient in all seed tissues, and *nced2 nced5 nced9* in which ABA is still produced in the endosperm. Reduced ABA levels were correlated with differences in the accumulation of transcripts associated with signaling networks, in addition to genes involved in seed maturation and desiccation tolerance. Furthermore decreased or increased ABA levels in biosynthesis (*qnced*) or catabolism (*cyp707a1 a2*) mutants modulated the oxidized proteome in dry seeds suggesting a link between the regulation of oxidative processes and dormancy depth by ABA.

**Auxin biosynthesis inhibitors, new tools for auxin study and regulation**

Kazuo Soeno[1], Yusuke Kakei[2] and Yukihisa Shimada[2]
*[1]Western Region Agricultural Research Center (WARC), National Agriculture and Food Research Organization (NARO), [2]Yokohama City University, Kihara institute for biological research*

Auxin is essential for plant growth and development, which makes it difficult to study the biological function using auxin-deficient mutants. Chemical genetics have potential to overcome this difficulty by transiently reducing the auxin action using inhibitors. In *Arabidopsis thaliana*, the indole-3-pyruvate (IPyA) pathway has been suggested as a major biosynthesis pathway of indole-3-acetic acid (IAA), the most common natural auxin. In this pathway, TRYPTOPHAN AMINOTRANSFERASE of ARABIDOPSIS1 (TAA1) catalyzes the first step of conversion from tryptophan (Trp) to IPyA, and then YUCCA, a flavin-containing monooxygenase, catalyzes the last step from IPyA to IAA. We developed several types of auxin biosynthesis inhibitors. The first group of compounds, targets TAA1, and contains aminooxy- and carboxy- groups. These compounds consist of novel compounds, designated ‘Pyruvamine (PVM)’. The second group of compounds targets YUCCA, and contains borate structure. These compounds inhibited the activity of recombinant enzymes *in vitro*, and reduced endogenous IAA content *in vivo*. Arabidopsis seedlings treated with inhibitors showed typical auxin deficient phenotypes and the growth inhibition was recovered in the presence of exogenous IAA. Enzyme kinetic studies of compounds revealed that they are competitive inhibitors of the substrate Trp or IPyA. Structure-activity

relationships of the compounds will be discussed. These small molecules will serve as novel and powerful tools in chemical genetics for study of auxin biology and regulation of auxin biosynthesis.
This work was supported by the Program for Promotion of Basic and Applied Researchers for Innovations in Bio-oriented Industry (BRAIN) to Y.S., JSPS KAKENHI Grant Number 26506016 to Y.K. and 26450046 to K.S. and the Scientific Technique Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry.

**11:00-12:35 pm Concurrent 5C: Hormones & Biotechnology**

**Plant Growth Regulators in Crop Production: Overview and New Developments**

Wilhelm Rademacher[1]
[1]*BASF SE, Global Research Crop Protection*

Products based on approximately 40 active ingredients are currently applied as plant growth regulators (PGRs) in agriculture, horticulture and viticulture. Typically, PGRs are represented by plant hormones or their synthetic analogs, by inhibitors of hormone biosynthesis or translocation, and by hormone receptor blockers. Many plant processes can be actively regulated with PGRs, e.g. acceleration or delay of seed germination, stimulation or reduction of shoot elongation, induction of flowering and fruiting, reduction or increase of fruit set, acceleration or delay of senescence processes including fruit ripening and defoliation. The achieved benefits range from facilitating crop management to increasing and securing yield and quality of the harvested produce and improving its storage and shelf life. The most widely used PGRs are (i) inhibitors of gibberellin (GA) biosynthesis, which are of particular importance to reduce the risk of lodging in wheat, rice and other cereal species and in oilseed rape, (ii) the ethylene-releasing ethephon, which is, inter alia, used to accelerate fruit ripening, to induce uniform flowering and fruit formation in pineapples, to increase latex production in rubber trees, and to advance boll opening in cotton, and (iii) GA3, which is applied to improve fruit quality in seedless table grapes, citrus, pears, and other species.
Current global annual sales of PGRs are in the range of US$ 1.4 billion, which represents approximately 2.5% of the crop protection market. Due to the relatively small market and high costs involved in finding, developing and registering new compounds, very few, if any, novel PGRs are expected to enter the market in the foreseeable future. The involved companies are rather concentrating on combining compounds already registered, improving formulations or finding additional uses for existing products.

**Transgenic alteration of ethylene biosynthesis and ethylene sensitivity increases grain yield in maize under filed drought-stress conditions**

Jeffrey E. Habben[1], Jinrui Shi[1], Rayeann L. Archibald[1], Xiaoming Bao[1], Nicholas J. Bate, Jason L. DeBruin, Dennis Dolan[1], Darren Hasegawa[1], Mark A. Chamberlin[1], Bruce J. Drummond[1], Timothy G. Helentjaris[1], H. Renee Lafitte[1], Nina Lovan[1], Hua Mo[1], Kellie Reimann[1], Jeffrey R. Schussler[1], Hongyu Wang[1], Ben P. Weers[1], and Robert W. Williams[1]
[1]*DuPont Pioneer*

Lack of sufficient water is a major limiting factor to crop production worldwide, and the development of drought-tolerant germplasm is needed to improve crop productivity. The phytohormone ethylene modulates plant growth and development as well as plant response to abiotic stress. Recent research has shown that modifying ethylene biosynthesis and signaling can enhance plant drought tolerance. First, a transgenic gene-silencing approach was used to modulate the levels of ethylene biosynthesis in maize (Zea mays) and determine its effect on grain yield under drought stress in a comprehensive set of field trials. Analysis of yield data indicated that transgenic events had significantly increased grain yield over the null comparators, with the best event having a 9 bushel/acre increase after a flowering period drought stress. Analysis of secondary traits showed that there was a consistent decrease in the anthesis-silking interval and a concomitant increase in

kernel number/ear in transgene-positive events versus nulls. Second, we discovered novel negative regulators of ethylene signal transduction in Arabidopsis (Arabidopsis thaliana) and maize. These regulators are encoded by the ARGOS gene family. In transgenic maize plants, overexpression of ARGOS genes reduces ethylene sensitivity. Moreover, field testing showed that UBIQUITIN1:ZmARGOS8 maize events had a greater grain yield than nontransgenic controls under both drought stress and well-watered conditions.

**Strigolactones: biosynthesis and potential in agriculture**

Christine Beveridge[1], Phil Brewer[1] and Kaori Yoneyama[2]
*[1]The University of Queensland, [2]Utsunomiya University*

Strigolactones have emerged as plant hormones with diverse roles in plant development. In addition to suppressing shoot branching/tillering they can suppress adventitious and lateral roots. They also enhance secondary growth and leaf senescence and can enhance the elongation of root hairs and primary root growth. Strigolactones play important roles in the rhizosphere where they promote symbioses that enhance nutrient and water uptake. On the negative side, strigolactones exuded from plants can stimulate plant parasites with potential to decimate crops. Over the eight years since discovery of genes for strigolactone biosynthesis, various researchers have mapped out a biosynthetic pathway and signal transduction system. This will be briefly reviewed along with our recent discovery of a new strigolactone biosynthesis gene and a discussion of the potential benefit strigolactone diversity may provide for plants and agriculture.

**Gibberellin Signalling: A Target For Improving Wheat Architecture**

Steve Thomas[1], George Lund[1], Archana Patil[1], Peter Hedden[1] and Andy Phillips[1]
*[1]Rothamsted Research*

Gibberellins (GAs) are plant hormones which control many aspects of plant growth and development, including stem elongation. The potential to improve crop traits by altering the GA signalling pathway is now well established. In wheat, the *Rht-1* semi-dwarfing mutations, which were instrumental in increasing yields during the Green Revolution, produce plants which are partially insensitive to GAs. This phenotype has a positive yield effect by reducing excessive stem elongation in response to the application of fertilizer and promoting increased partitioning of assimilate into the grain. DELLA proteins repress stem elongation and GAs relieve this repression by promoting the rapid degradation of DELLAs. The *Rht-1* semi-dwarfing mutations in wheat have been demonstrated to encode abnormal DELLAs. In work aimed at gaining a better understanding of how these alleles confer beneficial effects on wheat development, we have identified the mutations in multiple *Rht-1* dwarfing alleles and provided evidence that they enhance the stability of the protein by blocking binding to the GA receptor, GID1, leading to GA-insensitivity. We have recently devised a targeted strategy for identifying novel GA-insensitivity *Rht-1* mutations that potentially have improved traits compared to known alleles. This approach involved screening an EMS mutagenized wheat population by TILLING to identify missense mutations that alter conserved residues within the GID1 binding domain. A yeast-based interaction screen is being used to assess whether these mutations block binding to a GA-GID1 complex. In addition to identifying novel *Rht-1* semi-dwarfing alleles, this screen provides a potential explanation for the lack of *Rht-A1* dwarfing alleles being identified in wheat breeding programmes. In an attempt to identify other novel dwarfing genes, we are also targeting other phytohormone signalling components using a TILLING-based approach. These screens have resulted in the generation of a novel wheat GA biosynthetic mutant which has a semi- dwarf stature.

**New cytokinin derivatives for plant biotechnology, agriculture and cosmetics**

Magdalena Bryksová[1], Lenka Plačková[1], Adeyemi O. Aremu[2], Ponnusamy Baskaran[2], Aloka Kumari[2], Lucie Plíhalová[1], Lukáš Spíchal[1], Johannes Van Staden[2], and Karel Doležal[1]

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6-benzylaminopurine, an important and affordable cytokinin, is routinely used in plant micropropagation for its stimulatory properties. However, this compound and/or its endogenous metabolites can negatively influence shoot proliferation as well as rooting and acclimatization competency in some plant species (Werbrouck et al., 1996, Aremu et al., 2012). Based on our recent search for naturally occurring aromatic cytokinins in plants, we discovered several groups of their analogues with high activity in different cytokinin bioassays as well as their ability to activate cytokinin receptors and/or to inhibit cytokinin oxidases. The best compounds were tested during micropropagation and acclimatization processes of selected plant species. Subsequently, a wide range of endogenous plant hormones were quantified *in planta* during these experiments and compared in relation to cytokinins exogenously applied for their micropropagation efficiency. Results of this quantification study have been used to design a second generation of cytokinin derivatives, with improved metabolic properties.

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**Translating frost tolerant seed degreening from Arabidopsis to Canola**

Mendel Perkins[1], Logan Skori[1], Subramanian Sankaranarayanan[1] and Marcus Samuel[1]

*[1]University of Calgary*

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 drive 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 ectopically express ABI3 have been developed. These transgenics were able to degreen more completely than wild type following frost treatments. In spite of being expressed ectopically the downstream up-regulation of *SGR2* was restricted to seeds suggesting additional regulation in the leaves prevents *ABI3* overexpression from resulting in premature chlorophyll catabolism in leaves. In addition estimations of the transgenic lines growth characteristics and yield suggest an absence of deleterious pleiotropic effects.

**Saturday, June 25, 2016**

**9:00-10:30 am Plenary VI: Hormone Interactions**

**Translational regulation of plant hormone responses.**

Jose M. Alonso[1]
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Survival of plants greatly depends on the ability of these sessile organisms to tune their hardwired developmental programs to the constant changes in their environment. Although it is clear that plant hormones play a central role in this signal integration process, the exact molecular mechanisms involved are still largely unknown. Until recently, most studies have approached this question by examining the effects of different plant hormone regimens on transcript levels. Our recent work has taken advantage of the development of genome-wide translation profiling (the Ribo-seq) to uncover a novel level of regulation in the plant response to the hormone ethylene. Specifically, we have found that the signaling molecule EIN2 and the nonsense-mediated decay proteins UPFs play a central role in a previously uncharacterized ethylene-induced translational response. Currently, we are investigating the role of other plant hormones in gene-specific translational regulation. Our studies are uncovering new nodes of interaction between hormones, as well as the role of 3'UTRs and 5'uORFs in the regulation of plant responses to these key growth regulators.

**11:00-12:30 pm Plenary VII: Hormone Perception & Signaling**

**Constitutive auxin response in Physcomitrella reveals complex interactions between Aux/IAA and ARF proteins**

Meirav Lavy[1], Mike Prigge[1], Sibs Tao[1] and Mark Estelle[1]
[1]*University of California, San Diego*

The coordinated action of the auxin-sensitive Aux/IAA transcriptional repressors and ARF transcription factors produces complex gene-regulatory networks in plants. Despite their importance, our knowledge of these two protein families is largely based on analysis of stabilized forms of the Aux/IAAs, and studies of a subgroup of ARFs that function as transcriptional activators. To understand how auxin regulates gene expression we generated a *Physcomitrella patens* line that completely lacks Aux/IAAs. Loss of the repressors causes massive changes in transcription with misregulation of over a third of the annotated genes. Further, we find that the *aux/iaa* mutant is blind to auxin indicating that auxin regulation of transcription occurs exclusively through Aux/IAA function. We used the *aux/iaa* mutant as a simplified platform for studies of ARF function and demonstrate that repressing ARFs regulate auxin-induced genes and fine-tune their expression. Further the repressing ARFs coordinate gene induction jointly with activating ARFs and the Aux/IAAs.

**Chemical dissection of ABA receptor function**

Sean Cutler[1]
[1]*University of California, Riverside*

Agricultural productivity is dictated by water availability and consequently drought is the major source of crop losses worldwide. The phytohormone abscisic acid (ABA) is elevated in response to water deficit and modulates drought tolerance by reducing water consumption and inducing other drought-protective responses. The recent identification of ABA receptors, elucidation of their structures and understanding of the core ABA signaling network has created new opportunities for both chemical and genetic manipulation of water use. An unusually large family of receptors encodes ABA receptors and, until recently, it was unclear if selective or pan-agonists

would be required. Our recent identification of the selective agonist quinabactin has resolved this issue and defined the ABA receptor *Pyrabactin Resistance 1* (PYR1) and its close relatives as key targets for water use control. My seminar will discuss the structure and function of ABA receptors, our work developing synthetic ABA receptor agonists, and the use of orthogonal receptors to enable agrochemical control of water use in transgenic plants.

**Plant membrane receptor activation by shape-complementary co-receptor kinases**

Michael Hothorn[1]
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Plants have evolved unique membrane receptor kinases which control plant growth, development and interactions with other organisms. These receptors harbor leucine-rich repeat (LRR) ectodomains, which can sense rather different small molecule, peptide and protein ligands. I will compare the LRR receptor kinases BRI1 (which senses a growth-promoting steroid hormone) and HAESA (which senses an abscission-controlling peptide hormone) in mechanistic detail. I will present structural, biochemical and genetic evidence that the co-receptor kinase SERK1 contributes to specific ligand recognition and to receptor activation in both BRI1 and HAESA. Finally, I will discuss how formation of a different receptor – co-receptor signaling complexes at the plasma membrane can trigger specific signaling outputs in the cytoplasm.

**References:**

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Santiago J, Henzler C, Hothorn M (2013) Molecular Mechanism for Plant Steroid Receptor Activation by Somatic Embryogenesis Co-Receptor Kinases. ***Science*** 341:889-92