WORKSHOPS
WORK-01-01

CHROMATIN REMODELLING IN INDUCIBLE GENE EXPRESSION

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

WORK-01-02

THE EPIC-COGE BROWSER FOR ARABIDOPSIS EPIGENOMIC DATA

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

WORK-01-03

AN ENDOGENOUS MOBILE RNAI PATHWAY REQUIRED FOR STRESS RESPONSE IN PLANTS

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

WORK-01-04

EPIGENETIC REGULATION OF CAROTENOID BIOSYNTHESIS: IMPACTS ON PLANT DEVELOPMENT

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

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

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

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RNA-directed DNA methylation (RdDM) plays a fundamental role in gene regulation and plant defence against invasive DNA. RdDM is induced by 24-nt small interfering RNAs (siRNAs) which are loaded onto Argonaute 4 (AGO4) to form an effector complex. This effector complex is recruited to genomic loci through physical interaction with long non-coding RNAs (IncRNAs), which function as a scaffold to determine the exact target genomic region at which a DNA methylation enzyme catalyses cytosine methylation resulting in de novo DNA methylation and gene repression. While 24-nt siRNAs have been well identified, IncRNAs have not been well characterised which has hindered the identification of RdDM-regulated genes in plants. Using nuclear RNA immunoprecipitation and Illumina sequencing, we constructed a highly enriched library and obtained sequences of ncRNAs specifically associated with AGO4. Comparison of AGO4-associated ncRNAs with microarray expression data of RdDM mutants identified novel protein coding gene targets of RdDM. Surprisingly, a large proportion of these potential RdDM target genes were down-regulated in RdDM mutants suggesting that they are normally activated by RdDM. These RdDM-activated genes are more enriched than the RdDM-repressed genes for AGO4-associated ncRNAs derived from the gene body. Thus, in addition to its canonical function in gene repression, RdDM may play a role in maintaining or activating gene expression, possibly by directing gene body methylation. Functional classification of these RdDM-activated genes show an over-representation of stress-responsive genes, many of which are induced upon infection by the fungus, Fusarium oxysporum. We propose that the RdDM pathway functions to maintain the expression of stress response genes to confer disease resistance. Grant acknowledgement: Australian Research Council Future Fellowship (FT0991956).
WORK-02-01

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

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

WORK-02-02

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

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

WORK-02-03

THE ROLE OF AUXIN IN THE MITOCHONDRIAL STRESS RESPONSE

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

WORK-02-04

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

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Glutaredoxins (Grx) are small ubiquitous enzymes, generally involved in re-reduction of diverse oxidative modifications using glutathione. In recent years data has accumulated about the important role Grxs play in the cellular redox network, regulating the activity of key enzymes in the Calvin cycle and TCA cycle, while during stress situations they can regenerate antioxidant enzymes such as peroxiredoxins or low molecular weight antioxidants such as dehydroascorbate. In order to gain a better understanding of how energy metabolism and redox regulation mediated by Grxs are linked, mitochondria are of specific interest. Despite evidence for multiple Grxs in Arabidopsis mitochondria, combined analysis of subcellular localisation of GFP fusion proteins and proteomics of isolated mitochondia has shown that there is really only one key player, GrxS15. In order to uncover the impact of GrxS15 in Arabidopsis mitochondria different in vitro and in vivo experimental approaches were employed. A GrxS15 T-DNA insertion line as well as an RNAi line show altered expression patterns and subsequently lead to a knockdown at the protein level. The reduction of GrxS15 amount results in a growth phenotype with significantly shorter roots compared to wildtype plants. This phenotype renders plants more susceptible to arsenic poisoning. Different forms of arsenic promote the generation of an even stronger root growth phenotype and the change of the overall root system development. Furthermore, the transgenic lines show smaller rosettes compared to wildtype plants. These results indicate that mitochondria are involved in the detoxification of the arsenic compounds, and moreover reveal that GrxS15 is a crucial element within this pathway. Taken together these findings have greatly improved the current understanding of Grx function in plant mitochondria.
WORK-03-01

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

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

WORK-03-02

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

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

WORK-03-03

THE EPIC-COGE BROWSER FOR ARABIDOPSIS EPIGENOMIC DATA

Gregory B.D.1, Bomhoff M.2, Li F.1 and Lyons E.2
1Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA. 2Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA.

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

WORK-03-04

POSMED: ANOTHER GATEWAY TO THE AIP DATABASES FROM LITERATURE

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

WORK-03-05

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

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

WORK-03-06

MINING POST TRANSLATIONAL MODIFICATIONS IN ARABIDOPSIS USING THE MODHUNTER

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The past decade has seen the development of a plethora of online proteomics resources in Arabidopsis reflecting multiple large-scale studies. These resources exist independently and lack a level of integration. The Multinational Arabidopsis Steering Committee, Proteomics (MASCP) has addressed this issue through the development of a proteomics aggregation portal, MASCP Gator (http://gator.masc-proteomics.org/). The portal provides a summary of proteomics and protein information aggregated directly from ten online resources. The development of this portal has enabled us to develop a bioinformatics technique to identify likely regions of post-translational modifications in proteins of Arabidopsis. The ability to locate and identify post translational modifications experimentally by mass spectrometry is extremely challenging and there is a requirement for complementary techniques. Virtually all large-scale proteomics analyses in Arabidopsis have identified proteins with unmodified peptides. Collectively, these data reveal modified regions of a protein as unmatched areas within a protein model. Using a recent large-scale N-linked glycosylation survey as a test set, we could demonstrate that unmatched regions represent modification hotspots in proteins. These sites can be further targeted for investigation and characterization. We have now developed a method to locate putative regions with modifications by exploiting mass spectral data in the public domain and are attempting to develop this into a functional portal for the assessment modifications in proteomic datasets.
**WORK-04-01**

**DYNAMICS GAINED FROM FLUORESCENT PROTEIN TECHNOLOGIES**

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

**WORK-04-02**

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

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

**WORK-04-03**

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

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

**WORK-04-04**

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

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Brassinosteroids (BRs) are plant hormones that are perceived by plasma membrane (PM)-located receptors such as Brassinosteroid Insensitive 1 (BRI1) in Arabidopsis thaliana. Additionally, BR signalling is also dependent on the function of the Somatic Embryogenesis Receptor-like Kinases (SERK) co-receptor family. Transgenic plant lines expressing BRI1-GFP and SERK3-mCherry were generated and analyzed by confocal microscopy and FRET-FLIM to visualize the molecular events upon initiation of BR signaling. In accord with the current model of BR signal transduction, a time-dependent and ligand-induced hetero-oligomerization between BRI1 and SERK3 was observed, similar to previous reports using immunoprecipitation. In addition, the spatially resolved FLIM images enabled us to localize these BRI1-SERK3 receptor complexes to restricted areas within the PM of live epidermal root cells, a cell file known to exhibit active BR signaling. In contrast to the established BRI1 signaling model, FRET-FLIM revealed that a substantial amount of the BRI1-SERK3 hetero-oligomers was preformed.
WORK-05-01

miRNA EVOLUTION IN THE CAMELINEAE

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

WORK-05-02

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

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

WORK-05-03

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

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In both plants and animals, elucidation of miRNA (miRNA) function through a traditional loss-of-function approach has proven difficult due to extensive genetic redundancy among most miRNA families. To address this, technologies such as target Mimics (MIMs) in plants or molecular SPONGES (SPs) in animals have been used as transgenic approaches to generate loss-of-function phenotypes. They overcome redundancy by sequestering highly similar miRNA family members, thereby perturbing endogenous miRNA-target interactions. Here, we test whether SPs can inhibit miRNA activity in plants. Synthetic SPs transcripts with 15 miRNA binding sites separated by four nucleotide spacers and driven by the 35S promoter were designed to individually target the Arabidopsis miR159 and miR165/166 families. While SPs with wild-type miRNA binding sites have no apparent impact on miRNA regulation, SPs containing miRNA binding sites with two central mismatches (cmSPs) can generate very strong loss-of-function phenotypes. Comparison of the efficacy of the cmSPs to the corresponding MIMs finds that the efficacy of the different approaches varies: SP165/166 appears much stronger than MIM165/166, whereas MIM159 can generate stronger phenotypes than cmSP159. However, analysis suggests that MIM159 targets both miR159 and the closely related miR1391 family, whereas cmSP159 appears specific for miR159. Therefore, in terms of efficacy and specificity, the use of cmSPs appears a practical technology for inhibiting miRNA function in plants. We argue that a key component of efficacy will be target site accessibility, and the use of multiple miRNA binding sites within a single transcript may be beneficial in this regard.

WORK-05-04

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

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Many plant responses are mediated by interactions between intracellular calcium and the second messenger cGMP formed by guanylate cyclases (GCs). Previously we identified a novel class of receptor-GCs containing the GC catalytic center embedded within the kinase domain and showed that the recombinant cytoplasmic domain of phytosulfokine receptor AIPS KR1 has both guanylate cyclase and kinase activity in vitro (Kwezi et al. 2011 J Biol Chem 286: 22580-8). We now show that physiological increases in calcium levels enhance GC activity of AIPS KR1 whereas these calcium levels reversibly inhibit kinase activity. In addition PSKR1 kinase activity is reduced in the presence of the GC product cGMP. Recombinant AIPS KR1 can undergo in vitro autophosphorylation and we have confirmed it has 14 phosphorylation sites in its cytoplasmic domain including 8 serine, 3 threonine and 3 tyrosine residues. Three phospho-serine residues at the juxta-membrane position were mutated to either mimic phosphorylation on or off states. Kinase activity was enhanced in the on mutant and suppressed in the off mutant while GC activity was unaffected suggesting calcium acts as a molecular switch of PSKR1-mediated signalling that can be modulated by the phosphorylation state. The challenge now lies in understanding how molecular interactions between the GC and kinase domains are capitalized on in the plant.
WORK-05-05

ANCESTRAL FUNCTION OF CLE SIGNALING IN LAND PLANTS

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

WORK-05-06

REGULATORY PEPTIDES THAT CONTROL ROOT DEVELOPMENT IN RESPONSE TO ENVIRONMENTAL CUES

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Plant root architecture requires coordinated regulation of endogenous developmental programs and environmental stimuli. It is known that phytohormones control many aspects of root development, however it has only recently been reported that small secreted peptides are implicated in aspects of root development including meristem maintenance, gravitropism and lateral root development. Here, we describe a regulatory peptide that affects several aspects of root development and is induced in the root tip by environmental cues, particularly nitrate starvation and high salt. Upon overexpression, or exogenous application of the peptide, there is a strong reduction in the overall size of the root system. Lateral root formation is perturbed at an early stage and primary root growth is dramatically slowed. A T-DNA insertion mutant shows the opposite phenotype, producing a larger root system, particularly under nitrate limitation and salt stress. Our work suggests a role for this peptide as a negative regulator of root development and provides a link between developmental programs and environmental stimuli.
SO MANY SMART WAYS TO DIE – PROGRAMMED CELL DEATH IN PLANTS
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Death of individual cells or cell populations is crucial for life and development of complex multicellular organisms. Cell death decisions are tightly regulated by complex molecular mechanisms generally referred to as programmed cell death (PCD) control. In animals, the molecular control of PCD has been intensively studied to better understand PCD-related diseases such as cancer, autoimmune defects, and neurodegenerative diseases. In plants, however, little is known on PCD mechanisms so far, though correct prevention or execution of PCD is critical for plant development and for the plant’s interaction with its environment. In this workshop we gather international experts of Arabidopsis PCD research in different cellular and biological contexts: PCD processes elicited by biotic and abiotic interactions with the environment, as well as PCD instances that are part of the plant’s developmental program. In my introductory presentation, I will give a short glimpse on recent progress in PCD research and PCD concepts in plants.

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

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

STAX, A NOVEL NEGATIVE TRANSCRIPTIONAL REGULATOR OF ARABIDOPSIS LEAF SENESCENCE
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Leaf senescence is a highly regulated, systematic process with great impact on yield, biomass and nitrogen partitioning. The process is basically mediated by developmental age; however, it is additionally influenced by an array of internal and environmental signals. Delayed senescence, accompanied by an extended period of photosynthesis, is often coupled with elevated stress tolerance and/or higher biomass accumulation. Thus, the timing of senescence is crucial in determining crop yield, having great agricultural importance. Our group studies the function of senescence-associated transcription factors (TFs) in order to unravel the complex regulatory mechanisms underlying the onset and progression of leaf senescence. Recently, we identified a novel TF, called STAX, as a major regulator of leaf senescence in Arabidopsis thaliana. STAX expression is enhanced during age-dependent as well as dark- and salt-induced senescence. Overexpression of STAX results in extended life span, whereas its knock-out mutant shows accelerated senescence suggesting a negative regulatory role for STAX on leaf senescence. In addition to delayed senescence, STAX overexpressors displayed a significant delay in bolting and increase in leaf biomass. In order to understand the gene regulatory network controlled by STAX, its binding site was identified. Using estradiol-induced overexpression of the STAX TF in combination with microarray-based transcriptome profiling (using Affymetrix ATH1 arrays) we were able to identify genes rapidly responding to enhanced STAX expression, representing candidate direct target genes. Taken together, our data suggest STAX as a key regulator of plant growth and development, including leaf senescence.
VND7-BINDING SEQUENCES REVEALED BY FLUORESCENCE CORRELATION SPECTROSCOPY

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

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

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

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

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TrayScan was developed from a partnership between CSIRO HRPPC and Photon Systems Instruments (Czech Republic) as a high throughput phenotyping platform designed specifically for monocot and dicot model plants and small seedlings. TrayScan can scan up to 15 trays of 20 plants per load and estimate within minutes, architectural parameters such as projected leaf area from 3 positions in space and also access physiologically relevant indicators such as canopy temperature and chlorophyll fluorescence. This is made possible by the use of several RGB cameras, thermal infrared (FIR) and a chlorophyll fluorescence imaging system. The platform allows controlling light and temperature conditions to pre-acclimatise the plants, is fully programmable and automated for the data acquisition process. Plants and trays are bar-coded, data and metadata are stored on a data base for subsequent analysis and mining. We illustrate here the performance of the platform using Brachypodium distachyon, a model plants for grasses and cereals. The genome of Brachypodium distachyon has been fully sequenced and mining. We illustrate here the performance of the platform using visible stereoscopy and infrared imaging, to monitor the growth and function of 320 plants at once. The system enables one to scrutinize plant growth with high spatial and high temporal resolution up to four times per hour, day and night, seven days a week. The growth chamber, in which the robot operates, is also equipped with a multi-wavelength LED-light enrichment system, thus providing a high level of spectral control. The TrayScan™ platform is an automated phenotyping platform equipped with a conveyor system enclosed in an acclimation room for allowing control of light and temperature. This system has a throughput of 2400 plants per day, is equipped with an automatic watering station for applying controlled drought stress, and provides image data from infrared, visible and pulse modulated chlorophyll fluorescence cameras.

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

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

WORK-07-05

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

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

WORK-08-01

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

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

WORK-08-02

ARABIDOPSIS DETECTIVES: INNOVATIVE APPROACH TO RESEARCH-LED DRIVEN TEACHING

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

FRIDAY

WORK-09-01

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

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

WORK-09-03

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

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Sulfur is essential for plant growth and fitness. The metabolic pathways for sulfate assimilation are regulated by supply of sulfate and demands for production of reduced sulfur compounds in plants. They are suggested to be regulated homeostatically under highly controlled mechanisms in which the functions of a central transcriptional regulator, SLIM1, and its downstream target genes are involved. In Arabidopsis, SLIM1 is primarily responsible to induce mRNA accumulation of sulfate transporters facilitating the import of sulfate across the plasma membranes in root epidermal and cortical cells and releasing sulfate from the vacuoles under sulfur-limited conditions. SLIM1 can also induce accumulation of microRNA-395 (miR395) which specifically targets on mRNAs encoding plastid-localizing ATP sulfurylase (ATP5, ATP3 and ATP4) and low-affinity sulfate transporter, SULTR2.1, and abolishes their functions in sulfur metabolism and internal sulfate translocation in response to sulfur deficiency. It is suggested that miR395 essentially limits the flux of sulfate assimilation in plastids and helps plants to allocate sulfate to source leaves. In this workshop, we will present the results from our recent study on metabolic flux regulation of sulfate assimilatory pathways and its relationships with transcriptome-wide responses of sulfur-regulated gene networks in Arabidopsis.

WORK-09-02

REGULATION OF HIGH AFFINITY PHOSPHATE TRANSPORTERS IN ARABIDOPSIS

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Phosphate (Pi) is a crucial and often limiting nutrient for plant growth. It is also a very insoluble ion heterogeneously distributed in soil. The uptake of this element relies on the presence of multiple high affinity transporters (PTH1 family) located in plasma membranes (1). Multiple and complex steps of regulation of these proteins were identified illustrating the capacity for plants to tightly control the level of these transporters into the cells (2,3,4,5). Radiosotope live micro-imaging system (6) was used to image spatial distribution of Pi absorption along the root. Combined with various genetic manipulation approaches to manipulate this broad family of transporters, it reveals unexpected location of absorption and provides opportunity to dissect the function of redundancy of this multigene family. 1 Nussaume et al. (2011). Front Plant Sci. 2 Misson et al. et al. (2004). Plant Mol Biol. 3 Bayle et al. (2011). Plant Cell 4 Thibaud et al., (2010). Plant J. 5 Misson et al. (2005). PNAS USA. 6 Kanno et al. (2012) Philos Trans R Soc Lond B Biol Sci.

WORK-09-04

NITRATE SENSING AND SIGNALING IN ARABIDOPSIS THALIANA

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Nitrogen (N) is one of the key mineral nutrients for plants and its availability has a major impact on their growth and development. The main sources for terrestrial plants in soils is nitrate (NO3−) and most often it is a growth limiting factor. Plants are able to sense NO3− in their environment, allowing them to quickly respond to the dramatic fluctuations of its availability. Significant advances have been made during the recent period concerning the molecular mechanisms of NO3− sensing and signalling in higher plants. The striking action of NO3− as a signal molecule on genome expression has been unravelled. Nevertheless, NO3− sensing systems have not been identified. These unexpectedly correspond to membrane transporters also ensuring the uptake of NO3− into root cells, thus generalizing the nutrient ‘transceptor’ (transporter/receptor) concept defined in yeast. Furthermore, components of the downstream transduction cascades, such as transcription factors or kinases, have also been isolated. Recently, a major breakthrough arising from this improved knowledge is a better understanding of the integration of NO3− and hormone signalling pathways, that explains the extraordinary developmental plasticity of plants in response to NO3−.
WORKSHOPS

WORK-09-05

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

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

WORK-09-06

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

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Forward and reverse genetics have accelerated the number of functional gene annotations made. However, signal transduction mechanisms, subjected to post-translational modifications, are much more tedious to decipher using these methods. One alternative approach is to profile the metabolite complement of the cell. As the chemical modulators in metabolism, it provides a more comprehensive overview of post-transcriptional and –translational events associated with the early phosphate starvation response. Here an unbiased, untargeted metabolome approach using a range of extraction and mass spectrometry methods was employed to monitor the chemical response upon phosphate starvation and re-supply conditions in Arabidopsis and rice. Metabolites analyses, combined with computational and empirical approaches have uncovered i) specific underlying patterns, ii) altered progression and iii) unique dynamics of the phosphate-responsive metabolome. Chemical annotation and functional characterization of these metabolites have been performed. As proof of concept, phosphate friendly 1, a metabolite that accumulate upon phosphate starvation, alter the root system architectural (RSA) response under phosphate sufficient conditions to elicit the same type of response as those roots grown upon phosphate starvation. Furthermore, phosphate friendly 1also alter RSA responses during N limitation. The results will be discussed in the context of the elucidation of the biosynthetic pathway of phosphate friendly 1 in plants, as well as the transcriptional and post-transcriptional regulators identified thusfar. This work provides novel insight into root growth and development underlying nutrient acquisition strategies.
WORK-10-01

INTRODUCTION: PROTEOMIC RESOURCES AND THE ARABIDOPSIS PROTEOMICS SUBCOMMITTEE

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

WORK-10-02

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

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Abscisic acid (ABA) is a phytohormone that regulates diverse plant processes, including seed germination and the response to dehydration. In the major ABA signaling pathway, three members of SNF1-related protein kinase 2 (SnRK2) family transmit ABA-induced signals through phosphorylation of downstream substrates. To identify such substrates, we screened thousands of phosphoproteins in Arabidopsis by mass spectrometry-based phosphoproteomics. We identified proteins that were phosphorylated in Arabidopsis wild-type plants, but not in mutants lacking SnRK2s (srk2dei), treated with ABA or subjected to dehydration stress. Comparative analysis revealed that 35 peptides were differentially phosphorylated in wild-type but not in srk2dei plants. Biochemical and genetic studies of candidate SnRK2-regulated phosphoproteins showed that SnRK2 promoted the ABA-induced activation of MAPK(s), AIMPK/1/2; that SnRK2 mediated phosphorylation of Ser65 in a bZIP transcription factor, AREB1, and stimulated ABA-responsive gene expression; and that a previously unknown protein, SnRK2-substrate 1 (SNS1), was phosphorylated in vivo by ABA-activated SnRK2s. Reverse genetic analysis revealed that SNS1 acts as a negative regulator of ABA responses. Thus, by integrating genetics with phosphoproteomics, we identified multiple components of the ABA-responsive protein phosphorylation network. (Umekawa et al. Sci. Signal. 6: rs8, 2013).

WORK-10-03

REDOX-REGULATION OF THE SUMO E2 IN PLANT IMMUNITY

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

WORK-10-04

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

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

WORKSHOPS

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