POSTERS

Wednesday - Friday

POS-WED-001

QTL ANALYSIS OF THE STAY-GREEN TRAIT USING A RECOMBINANT INBRED LINE POPULATION IN SORGHUM

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The stay-green trait is a mechanism by which plants adapt to post-flowering drought stress thus enhancing continued photosynthesis and grain filling, leading to higher yields and biomass. Our study aims at identifying stay-green QTLs at the seedling stage and characterizing the genes responsible for it in sorghum. 252 Recombinant inbred lines (RILs) were derived from a cross between BTx623, the genome reference sequence possessing stay-green characteristics; and a non stay-green Japanese landrace, NOG. The F6 population was used to obtain high-resolution genotyping data with >3,700 markers via RAD sequencing. Comparative analysis of chlorophyll content readings taken from the second and third leaves of 24-day-old seedlings grown in a controlled growth chamber, allowed us to identify seven stay-green QTLs. Two QTLs on chromosomes 2 and 6 were found to be consistent in two replications, and these also explained the highest phenotypic variation among all the QTLs. Additional QTLs obtained from leaf 2 were located on chromosomes 1, 6 and 8, while measurements from leaf 3 yielded additional QTLs on chromosomes 1, 2, 3, and 7. The total phenotypic variation explained by the QTLs obtained from chlorophyll measurements of the second and third leaves were 19% and 23%, respectively. The presence of two consistent QTLs suggests that these might be major QTLs involved in the expression of the stay-green trait in our sorghum lines. Further genotypic analysis on these RILs is underway to attain a population that can be used for linkage analysis and moreover, field trials to complement this data are currently ongoing.

POS-THU-002

MOLECULAR AND PHENOTYPIC CHARACTERIZATION OF VITAMIN C ENRICHED RICE

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Human iron (Fe) deficiency is the most common nutritional deficiency worldwide affecting over 2 billion people. Enhancing the concentration and/or bioavailability of Fe in staple crops through conventional plant breeding or modern biotechnology, a process known as biofortification, represents a sustainable strategy for increasing human Fe intakes. Ascorbic acid (AsA), commonly known as vitamin C, is a strong enhancer of Fe bioavailability, however most staple crops have low AsA content. Notably, the active form of AsA is not detectable in polished and whole grain rice. Increasing AsA concentration in rice may therefore represent an effective and novel means of enhancing Fe bioavailability. Moreover, AsA is a major antioxidant in plants capable of preventing or minimizing the damaging effects of reactive oxygen species produced by abiotic stress. The major abiotic stresses limiting rice production worldwide such as drought, heat, and salinity are predicted to increase in frequency due to climate change. The need for crops with broad abiotic stress tolerance is thus critical for ensuring global food security. This project aims to characterize rice plants overexpressing the *OsGGP* gene, encoding the enzyme GDP-L-galactose phosphorylase, which regulates the rate limiting step in AsA biosynthesis. Preliminary analyses have found that T_1 rice plants overexpressing *OsGGP* produce up to five-fold more AsA than wild-type rice in imbibed grain and that many lines have increased height compared to wild-type rice under normal growth conditions. Here we present a molecular and phenotypic characterization of the *OsGGP* overexpressing rice plants including transgene copy number, transgene expression analysis, and AsA concentration in vegetative tissues.

POS-FRI-003

TWO PHOSPHOETHANOLAMINE METHYLTRANSFERASES SYNERGISTICALLY REGULATE ROOT DEVELOPMENT AND FERTILITY IN ARABIDOPSIS

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Phosphatidylcholine is a major phospholipid of cellular membranes. Its biosynthetic pathway is complex but in plants the sole entry point for de novo phosphatidylcholine synthesis is thought to be the conversion of phosphoethanolamine to phosphomethylethanolamine catalysed by phosphoethanolamine N-methyltransferase (NMT). The Arabidopsis genome is predicted to encode three NMTs. One of these enzymes (NMT1) has been characterized[1] and confirmed to catalyse all three methylations from phosphoethanolamine to phosphocholine, while a second one was reported to only catalyse the last two methylations from phosphomethylethanolamine to phosphocholine, and hence re-annotated as a PMEAMT[2]. We will report on the biochemical and *in planta* function of the third enzyme (NMT3), which was so far unknown. Similar to NMT1, NMT3 restores the growth of a yeast choline-auxotroph strain, and suppresses the root defects of the Arabidopsis *xipot1/nmt1* loss-of-function mutant, when driven by *NMT1* promoter. However, contrary to that mutant, the *nmt3* knock-out mutant is phenotypically similar to wild-type under normal growth conditions. In stark contrast, the *nmt1 nmt3* double mutant shows drastically reduced growth of both root and rosette, delayed bolting and a bushy, ball-like inflorescence, with dramatically reduced fertility. Loss of both *NMTs* impacts on cell proliferation and expansion, impairs the development of male and female floral organs, and also affects embryogenesis. Our results demonstrate unequal contributions of the two *NMTs* in these phenotypes, with gene dosage effects, and also specificity brought about by non-totally overlapping expression patterns. They highlight the physiological importance of the methylation pathway for sustaining plant growth and reproduction. 1. Cruz-Ramírez et al. (2004), Plant Cell: 16, 2020-2034. 2. BeGora et al. (2010) JBC, 285: 29147-29155.

POS-WED-004

FUNCTIONAL CHARACTERISATION OF GIBBERELLIC ACID IN SOYBEAN *(GLYCINE MAX)* NODULATION

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Legumes represent some of the most globally important food, feed and biofuel crops. Soybean (*Glycine max*) is the most economically significant legume species and is the third largest crop grown worldwide, following rice and wheat. Like most legume species, soybean is characterised by its ability to form root nodules in response to compatible nitrogen-fixing rhizobia bacteria (*Bradyrhizobium japonicum*). Nodule formation requires complex molecular mechanisms involving an array of signals, including the phytohormone gibberellic acid (gibberellin; GA). Nodule development is also tightly regulated by the host plant through additional signalling mechanisms. GAs appear to be required at different stages of nodulation, with their concentration highly regulated to achieve successful nodulation. Indeed, a window of GA appears to be required temporally and spatially, with too much or too little being inhibitory to nodule development. High throughput RNA-seq identified a set of differentially expressed GA oxidase genes in soybean. *GmGA20ox a*, *GmGA3ox 1a*, and *GmGA2ox* were all up-regulated in soybean roots inoculated with compatible rhizobia, indicating a requirement for these genes in nodulation; however, their precise roles in nodulation remain unclear. Our research aims to enhance the knowledge of GAs, and the individual GA oxidase genes, in soybean nodulation. Findings from our work will be presented.

POS-THU-005

INDUCTION OF COMPRESSION WOOD BLOCKS DEVELOPMENT OF SPIRAL GRAIN IN RADIATA PINE

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Spiral grain is the systematic deviation of wood grain away from the stem axis, and is problematic for the timber industry as cut timber with grain exceeding 5o can develop a twist. Grain in radiata pine, like most gymnosperms, is initially vertical but develops a left-handed helix during the first years of growth, often reaching 10o from vertical. In subsequent years, the direction of grain change reverses, and grain angle decreased and often becomes right-handed. The mechanisms of spiral grain formation, which presumably require the rotation of cambial initials, remain poorly understood. We have used ImageJ to measure spiral grain development in young, vertically-grown radiata pine trees through multiple, independent approaches. Resin canals (running parallel to grain) were imaged in serial sections with circularly-polarised light, appearing as black spots against birefringent tracheid walls, and were reconstructed and measured following image alignment. Grain was also measured from Fast Fourier Transforms of tangential longitudinal sections derived from X-ray microtomography. These experiments confirmed that left-handed spiral grain was initiated in the first year of growth and only several millimetres outside the central pith, and that grain angles steadily increase after that. In tilted trees, however, the induction of compression wood on the lower-side of the tree interfered with spiral grain development, with grain in these regions failing to become more left-handed, although spiralling developed normally on the upper side. This asymmetry in grain development between the upper and lower sides of the same stem confirms the link between spiral grain and compression wood formation, and suggests an entry point through which the mechanisms that generate spiral grain might be studied.

POS-FRI-006

CHARACTERIZATION AND FUNCTIONAL ANALYSIS OF CALMODULIN BINDING RECEPTOR-LIKE CYTOPLASMIC KINASE 3, CRCK3 IN *ARABIDOPSIS THALIANA* (L) HEYNH

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Receptor-like cytoplasmic kinases (RLCKs) are plant specific proteins that belong to largest protein family of receptor-like kinases (RLKs). Normally, RLCKs integrate with RLKs to regulate a variety of biological processes from plant growth and development to stress responses. Nearly 150 RLCKs encoded in Arabidopsis genome, however, only few of them have been characterized. We have identified a RLCK with pathogenesis associated expression pattern. It was previously described as a calmodulin binding domain receptor-like cytoplasmic kinase 3 (CRCK3), but was not characterized. The obtained data show that loss-of-function crck3 mutants were compromised in defence against Fusarium oxysporum and have altered expression levels of marker defence related genes. Interestingly, crck3 mutants display reduced reactive oxygen species (ROS) production, callose deposition in response to treatment with flagellin peptide (flg22) and elongation factor-Tu peptide (elf18). These data indicate that CRCK3 is involved in both early and late plant defence responses. Analysis of subcellular localization of CRCK3-GPF in Arabidopsis mesophyll protoplasts revealed fluorescence at the plasma membrane, implicating interaction with membrane bound proteins, possibly RLKs. Taken together, our data firmly established a role of CRCK3 in plant innate immunity.

POS-WED-007

ROLE OF LEUNIG AND LEUNIG_HOMOLOG IN REGULATING EARLY EMBRYONIC PATTERNING IN ARABIDOPSIS

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The closely related transcriptional regulators LEUNIG (LUG) and LEUNIG_HOMOLOG (LUH) are recruit to chromatin through interactions with the SEUSS (SEU) and SEU-LIKE (SLK) co-regulators and transcription factors. Work from our group and others has shown that the LUG regulatory complex plays diverse roles in Arabidopsis development including the regulation of leaf development and shoot apical meristem activity and the regulation of floral organ identity. Given the similarity in protein structure and expression profiles, it has been suggested that LUG and LUH might perform redundant functions. To explore this possibility, we generated *lug luh* double mutants and found that they display severe embryonic patterning defects. Furthermore, analysis of cell-type specific and auxin patterning markers reveal that apical-basal patterning and auxin localization are not correctly established during the early stages of *lug luh* embryo development. Moreover, finding similar defects in *seu slk1 slk2* triple mutant embryos suggests that there is a high degree of redundancy between LUG/SEU/SLK and LUH/SEU/SLK complexes during embryogeneis. Genome-wide interactome studies have previous shown that SLK2 physically interacts with the WUSCHEL-RELATED HOMEOBOX9 (WOX9) transcription factor. We not only confirm this interaction, but show that other members of the SEU/SLK family interact with WOX9, as well as the closely related WOX8 protein. These interactions are highly significant, as previous work from several groups have shown that *wox8 wox9* double mutant embryos display apical-basal patterning defects and thus suggest that the WOX transcription factors may be part of the LUG/LUH regulatory complex. We report our latest findings that support the existence of this complex in plants.

POS-THU-008

ALLELIC VARIATION IN GRAPEVINE HKT CONTRIBUTES TO DIFFERENTIAL LEAF SODIUM EXCLUSION

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Salinity is a major problem for viticulture. Accumulation of sodium ions in berries can result in wine that is unpalatable, and accumulation in shoots negatively impacts on yield. We have identified a high affinity potassium transporter (HKT) from grapevine roots that is selective for sodium and no other monovalent cations when expressed in *Xenopus laevis* oocytes. We have cloned and functionally characterised four allelic variants of this HKT from two heterozygous grapevine rootstocks that possess contrasting salt tolerance. When expressed in *Saccharomyces cerevisiae*, two allelic variants strongly inhibited growth on high sodium medium, while another two allelic variants caused weaker growth inhibition. Furthermore, in Xenopus oocytes, we observed differences in the electrophysiological properties of the allelic variants. In the progeny of a cross between the two grapevine rootstocks, individuals that were homozygous for the toxic HKT allelic variants contained less shoot sodium, heterozygous individuals showed intermediate shoot sodium accumulation, and individuals homozygous for the less toxic HKT allele were less able to exclude sodium from leaves. These results indicate a role of this protein in the natural variation in shoot sodium exclusion across grapevine varieties, and provide novel insights into the mechanism of salinity tolerance mediated by HKT proteins in plants.

POS-WED-009

CIRCADIAN CLOCK REGULATION OF INNATE IMMUNITY IN PLANTS

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The circadian clock is an endogenous time-keeping mechanism that synchronises biological processes with the external environment, such that they occur at optimal times of the day While the clock has long been known to allow plants to anticipate predictable daily changes in abiotic stimuli, only recently has it become apparent that it also allows them to anticipate interactions with other organisms. We have demonstrated that the clock modulates the strength of the plant immune response against both the biotrophic bacterial pathogen *Pseudomonas syringae* and the necrotrophic fungus *Botrytis cinerea*; Arabidopsis displays differential susceptibility to these pathogens that is dependent on the time of infection, persists under constant light conditions, and is lost in arrhythmic clock mutants. As a first step towards understanding the molecular mechanisms by which the clock regulates plant innate immunity, we carried out transcriptome profiling of Arabidopsis following inoculation with *B. cinerea* spores at subjective dawn or night. We observed that decreased susceptibility to *B. cinerea* following inoculation at subjective dawn was associated with a more rapid and robust transcriptional regulation of the defence response, suggesting that the clock gates the expression of infection-responsive genes. Direct target genes of core clock regulators were also enriched among the transcription factors that responded more rapidly to infection at subjective dawn than subjective night, suggesting a direct influence of the clock on the defence regulatory network. Finally, jasmonate signalling plays a crucial role in the rhythmic susceptibility of Arabidopsis to*B. cinerea* as demonstrated by the loss of temporal variation in susceptibility in mutants defective in key transcriptional and post-transcriptional regulators in this hormone signalling pathway.

POS-WED-010

THE SINGLE EVOLUTIONARY ORIGIN OF CHLORINATED AUXIN PROVIDES A PHYLOGENETICALLY INFORMATIVE TRAIT IN THE FABACEAE

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Chlorinated auxin (4-chloroindole-3-acetic acid, 4-Cl-IAA), a highly potent plant hormone, was once thought to be restricted to species of the tribe Fabeae within the Fabaceae, until we recently detected this hormone in the seeds of *Medicago*, *Melilotus* and*Trifolium* species. Furthermore, we found no evidence that *Pinus* spp. synthesize 4-Cl-IAA in seeds, contrary to a previous report. The absence of 4-Cl-IAA in the seeds of the cultivated species *Cicer aeritinum* from the Cicerae tribe, immediately basal to the Fabeae and Trifolieae tribes, suggested a single evolutionary origin of 4-Cl-IAA. Here, we provide a more robust phylogenetic placement of the ability to produce chlorinated auxin by screening key species spanning this evolutionary transition. We report no detectable level of 4-Cl-IAA in *Cicer echinospermum* (a wild relative of *Cicer aeritinum*) and 4 species (*Galega officinalis*,*Parochetus communis*, *Astragalus propinquus* and *A. sinicus*) from tribes or clades more basal or sister to the Cicerae tribe. We did detect 4-Cl-IAA in the dry seeds of four species from the genus *Ononis* that are either basal to the genera *Medicago*, *Melilotus*and *Trigonella* or basal to, but still within, the Fabeae and Trifolieae (ex. *Parochetus*) clades. We conclude that the single evolutionary origin of this hormone in seeds can be used as a phylogenetically informative trait within the Fabaceae as well as an ideal model system to further investigate the action and activity of halogenating enzymes in plants.

POS-THU-011

IDENTIFICATION OF REGULATORY GENES CONTROLLING CELL WALL INVERTASE EXPRESSION IN ARABIDOPSIS REPRODUCTIVE ORGANS

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In plants, sucrose (Suc) is synthesised in source leaves and then translocated through the phloem to non-photosynthetic sink tissues. Upon reaching sink cells, Suc is often apoplasmically hydrolysed by cell wall invertase (CWIN) into glucose (Glc) and fructose (Fru) as building blocks, energy source and signalling molecules for growth, development and yield formation (1). Despite the central roles of CWIN in assimilate partitioning and sugar signalling, however, the upstream molecular machineries that control CWIN expression in plants remain unknown (1). This PhD project therefore aims to identify the upstream regulatory genes controlling CWIN expression in reproductive organs using *Arabidopsis thaliana* as a model plant. We hypothesised that CWIN gene expression is regulated by transcription factors (TFs) and small RNAs (sRNAs) at the transcriptional and post-transcriptional levels. Bioinformatics analysis was used to identify candidate regulatory genes encoding TFs or sRNAs that likely control the expression of *CWIN2* and *CWIN4*, which are highly expressed in *Arabidopsis* reproductive organs (flower and silique). As a result, 18 TF genes and one microRNA (miRNA), miR3932, were selected as putative regulators of *CWIN2* or *CWIN4* for further validation. Among the 18 TF candidates, ARF6, ARF8, AP3 and CRC were identified as putative regulators for CWIN gene expression based on the finding that *CWIN2* or *CWIN4* expression was significantly reduced in *arf6*, *arf8*, *ap3* and *crc* mutants. The miR3932, which matches *CWIN2* RNA sequence, was found to be expressed in a complementary fashion with *CWIN2*, indicating miR3292 may be involved in mediating *CWIN2* RNA degradation. In the future, a combination of molecular and biochemical approaches will be employed to further confirm the candidate regulators of *CWIN2* or *CWIN4*, and to examine the nature of their regulation of CWIN gene expression in *Arabidopsis* reproductive organs. (1) *Ruan Y-L 2014 Annu Rev Plant Biol 65, 33-67.*

POS-FRI-012

WALL INGROWTH DEPOSITION IN PHLOEM PARENCHYMA TRANSFER CELLS OF *ARABIDOPSIS* IS A NOVEL HETEROBLASTIC TRAIT UNDER CONTROL OF THE MIR156/SPL REGULATORY MODULE

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Transfer cells (TCs) *trans*-differentiate from existing cell types to facilitate enhanced membrane transport of nutrients at symplasmic/apoplasmic interfaces. The morphological specialization of TCs lies in their augmented surface area of plasma membrane resulting from intricate wall ingrowths. In *Arabidopsis*, phloem parenchyma (PP) TCs form from differentiated PP cells in vascular bundles of cotyledons, leaves and sepals. We report that PP TCs with extensive wall ingrowths are ubiquitous in juvenile leaves, but substantially less abundant in adult leaves, an observation consistent with PP TC development representing a novel trait of vegetative phase change (VPC) in Arabidopsis. Consistent with this conclusion, the abundance of PP TCs with extensive wall ingrowths varied across rosette development in three ecotypes that display different juvenile phase lengths, and extensive deposition of wall ingrowths was observed in rejuvenated leaves following defoliation. PP TC development across juvenile, transition and adult leaves correlated positively with levels of miR156, a major regulator of VPC in plants, and negatively with levels of miR156-targeted *SQUAMOSA PROMOTER BINDING PROTEIN LIKE* (*SPL*) genes. Corresponding changes in wall ingrowth deposition were observed when miR156 was overexpressed or its activity suppressed by target mimicry. Wall ingrowth deposition was reduced in plants carrying miR156-resistant forms of *SPL9*, *SPL10* and *SPL15*, and was increased in the double mutant spl9/spl15. Importantly, no change in xylem abundance was observed in these lines, indicating a VPC-specific wall ingrowth response. Collectively, our results point to wall ingrowth deposition in PP TCs being under control of the genetic program regulating VPC via the miR156/*SPL* module.

POS-WED-013

THE KEY DRIVERS OF CYCLOTIDE PRODUCTION IN BUTTERFLY PEA

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Butterfly pea (*Clitoria ternatea*) produces interesting disulfide rich head-to-tail cyclized peptides, called cyclotides. Butterfly pea is the first and only reported cyclotide-producing species from the Fabaceae family, an economically important family that includes vital crops such as soybeans and peanuts. Cyclotides are hypothesized to have evolved as means for plants to defend themselves from biotic factors. Their defensive powers are useful in the agricultural sector where a commercial butterfly pea product consisting of extracts containing cyclotides will soon be on the market. The cyclotides in butterfly pea are biosynthesised from precursors embedded within members of the albumin-1 multi-gene family. Although the pathway that matures cyclotides from these precursors is known from *in vitro* experiments, the factors that control the rates of cyclotide production *in planta* are unknown. Why they are constitutively produced in such great abundance is also not well understood. To shed light on these questions, we determined the cyclotide expression profiles of 150 butterfly pea plants at the peptide level. Here we show that different individuals variably express their cyclotide compliment. The major cyclotide of vegetative tissue, CterM, was found to be missing in a small number of seed-grown plants. The amino acid sequences of the CterM-negative plants contain non-silent mutations in their full length precursor albumin sequence, and these polymorphisms may contribute to the lack of mature CterM expression. Furthermore, promoter regions of the accessions in which CterM is either highly expressed or under-expressed are investigated to determine the potential regulatory elements that drive cyclotide expression. Together our evidence suggests butterfly pea cyclotides constitute a part of butterfly pea's innate defences.

POS-THU-014

RAPID METHYLATION OF TRANSGENES UPON PLANT TRANSFORMATION - A METHODOLOGY

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Genetic Modification, whereby foreign DNA (transgenes) is introduced into plants, serves multiple purposes in plant biology research and can improve or introduce favourable traits in many crop species. However, transgene expression is highly variable due to multiple and interacting factors, one of these is DNA methylation which results in transcriptional inhibition or 'gene silencing'. As a result, large numbers of plants must be screened to find stable transformants with consistent transgene expression. Finding ways to mitigate transgene methylation will be greatly beneficial to the plant transformation community. Here we present a methodology of an assay to rapidly assess transgene methylation. With this assay, variables such as intron inclusion within the transgene and 'knock-outs' of genes involved in the transcriptional gene silencing pathways can be rapidly tested within a transient plant transformation system. Transient co-infiltration in *Nicotiana benthamiana* was carried out using the eGFP reporter gene under the constitutive expression of the 35S promoter and a hairpin RNA targeting the eGFP transgene. Using this assay, we demonstrate accumulation of methylation over a relatively short time course with 50% methylation of all cytosine nucleotides at five days post infiltration in a region encompassing a 418 bp junction of the 35S promoter and eGFP transgene.

POS-FRI-015

SYNERGISTIC ROLES OF THREE RICE *(ORYZA SATIVA) ERECTA*-LIKE GENES IN THE REGULATION OF FLORAL ORGAN IDENTITY AND SPIKELET FERTILITY

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Rice spikelet development and fertility improving rice grain yield is a major focus of breeders around the world. The spikelet is the basal unit of the rice panicle and its correct development is crucial for reproductive success. We will report on the role of three*ERECTA/ERECTA*-like leucine-rich receptor-like kinases in rice spikelet formation. The Arabidopsis *ERECTA* gene family as a whole has been shown to regulate floral meristem function, inflorescence architecture and organ shape, but the role of its individual members remains unknown. Furthermore, *ERECTA* gene function has not been investigated at all in any other type of inflorescences. Our results reveal the synergistic roles of the three rice *ERECTA*-like genes in the regulation of floral meristem determinacy and floral organ identity, through partial redundancy as well as specificity among family members. Furthermore, we identify environmental factors that strongly modulate the function of rice *ERECTA* homologs in inflorescence development and fertility, with implications for the potential of these genes as tools for genetic improvement of floral development in rice and its adaption to environmental change.

POS-WED-016

DEVELOPING RICE AS A PRODUCTION SYSTEM FOR CYCLIC THERAPEUTIC PEPTIDES

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Plant-produced cyclic peptides range from 14~37 amino acids in length and are characterised by a head-to-tail cyclised backbone that is further strengthened by intramolecular disulphide bonds. They exhibit various bioactivities, including uterotonic, antimicrobial and anti-HIV activities, which are potentially exploitable for therapeutic uses. Moreover, due to their unique stable topological structure and tolerance to residue substitution, cyclic peptides have become promising molecular scaffolds for pharmaceutical protein-engineering applications. Currently, chemical synthesis is a common strategy for producing native and modified cyclic peptides, as well as recombinant synthesis and natural extraction. However, these strategies are limited to large-scale production by the high production cost, complexity of separation and deficient knowledge of the biosynthesis pathway. To address these issues, we are investigating a non-native cyclic peptides producing species, rice, as a plant bioreactor to express cyclic peptides of interest. Rice represents a promising recombinant peptide production platform based on its proven stable accumulation of proteins, low bio-safety risk and cost-effectiveness. So far, no native cyclic peptides have been identified in any monocot species, however, several peptide precursor genes with homology to cyclic peptide genes have been identified, differing only in the absence of residues known to be important for *in planta* cyclisation. In this study, we aim to test the capacity of rice to produce cyclic peptides through transformation of a variety of precursor peptide genes together with or without helper accessory genes that are believed to aid cyclisation *in planta*. It is envisioned that engineering rice to be a cyclic peptide bioreactor will enable rapid production of cyclic peptide pharmaceuticals in large quantity.

POS-THU-017

INTERACTIONS BETWEEN ETHYLENE, GIBBERELLINS, AND BRASSINOSTEROIDS IN THE DEVELOPMENT OF RHIZOBIAL AND MYCORRHIZAL SYMBIOSES OF PEA

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The regulation of arbuscular mycorrhizal development and nodulation involves complex interactions between the plant and its microbial symbionts. We used the recently identified ethylene-insensitive *ein2* mutant in pea to explore the role of ethylene in the development of these symbioses. We showed that ethylene acts as a strong negative regulator of nodulation, confirming reports in other legumes. Double mutants produced by crosses between *ein2* and the severely gibberellin-deficient *na* and brassinosteroid-deficient *lk* mutants showed increased nodule numbers and reduced nodule spacing compared with the *na* and *lk* single mutants, but nodule numbers and spacing were typical of *ein2* plants, suggesting that the reduced number of nodules in *na* and *lk* plants is largely due to the elevated ethylene levels previously reported in these mutants. We showed that ethylene can also negatively regulate mycorrhizae development when ethylene levels are elevated above basal levels, consistent with a role for ethylene in reducing symbiotic development under stressful conditions. In contrast to the hormone interactions in nodulation, *ein2* does not override the effect of *lk* or *na* on the development of arbuscular mycorrhizae, suggesting that brassinosteroids and gibberellins influence this process largely independently of ethylene.

POS-FRI-018

DEVELOPING PLANTS AS BIOFACTORIES FOR RE-ENGINEERED CYCLIC PEPTIDES

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For many therapeutic proteins, a plant-based production system offers a cost-effective and greener alternative to traditional synthesis methods. This was recently exemplified by the first plant-produced human therapeutic enzyme, taliglucerase alfa, which was FDA approved for the treatment of Gaucher's disease. In this study we aim to develop plants into biofactories for the production of valuable therapeutic peptides. Of particular interest is a class of natural plant-produced peptides termed cyclotides, which are post-translationally cyclized in planta and strengthened by a characteristic disulfide bond arrangement. Cyclotides are excellent scaffolds for stabilizing small peptides with therapeutic activity that would otherwise be unstable and unusable in a pharmaceutical sense. Recently, a cyclotide was re-engineered as a drug candidate for treatment of chronic myeloid leukemia (CML), a blood cancer prone to developing resistance to current treatments. Here, an engineered gene sequence encoding this cyclotide graft was transformed into the model plant *Arabidopsis thaliana* to establish the feasibility of plant-based production. Here, we present our progress in advances towards plants as biofactories for pharmaceutically relevant cyclic peptides. Analysis of peptide mass, cyclisation and yield was determined by MALDI-TOF-MS analysis and NMR. Our ultimate aim is to develop a flexible, costs effective, and scalable production option. This will require progression to commercially viable crop plants such as potatoes, which can then be used to take this biotechnology to people in the first and third world who can benefit from not only the medicines, but the ability to meet their own production needs.

POS-WED-019

A CALMODULIN-LIKE PROTEIN REGULATES FLG22-INDUCED PLASMODESMAL CLOSURE AND INNATE IMMUNE RESPONSES TO BACTERIA

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Plants sense microbial signatures via the activation of pattern recognition receptors (PPRs), which triggers a range of cellular defences. One response is the closure of plasmodesmata, which reduces symplastic connectivity and the capacity for direct molecular exchange between host cells. Plasmodesmal flux is regulated by a variety of environmental cues but the downstream signalling pathways are poorly defined, especially how cytoplasmic calcium regulates plasmodesmal closure. Here, we identify that flg22-induced closure of plasmodesmata is mediated by a plasmodesmal-localised, Ca^{2+}-binding protein Calmodulin-like 41 (CML41). CML41 mediates rapid callose deposition at plasmodesmata following flg22 treatment in a calcium-dependent manner, independently of other defence responses triggered by flg22 perception. CML41 transcriptionally responds to flg22 and is essential for full defence against *Pseudomonas syringae*.

POS-THU-020

THE EFFECT OF TARGET RNA ON SMALL RNA ACCUMULATION IN PLANTS

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RNA silencing is a fundamental plant defence and gene control mechanism in plants that are directed by 20-24 nt small interfering RNA (siRNA) and microRNA (miRNA). RNA silencing has been exploited to develop powerful gene silencing technologies such as the hairpin RNA (hpRNA) transgene technology. Since siRNAs are processed from long dsRNA by Dicer-like proteins, the expected ratio between sense and antisense siRNA strands should be 1:1. However, siRNAs from either the plant genome (e.g. hpRNA transgene) or from infecting viruses often show a strong unequal distribution of the sense and antisense strands. How the strand bias occurs in plants remains unclear, although studies in animal cells have implicated an involvement of target RNA in affecting small RNA accumulation. To investigate this, we used Arabidopsis and tobacco as model plants to examine the accumulation of hpRNA transgene-derived sRNA in the presence or absence of target mRNA. Results from transient expression using Agrobacterium infiltration showed that the presence of abundant target RNA affects the accumulation of anti-sense siRNA. To examine the effect of target RNA on siRNAs from stably integrated hpRNA transgenes, we generated plants expressing hpRNA alone or both hpRNA and target mRNA. These plants are being analysed for siRNA abundance, and results will be presented. Since siRNA polarity (sense or antisense), sequence composition and abundance are important for the ability of the siRNA to induce efficient silencing, understanding the factors that affect the siRNA abundance can potentially be used to improve the efficiency of gene silencing in plants.

POS-FRI-021

FUNCTIONAL ANALYSIS OF GENES INVOLVED IN THE REGULATION OF SOYBEAN NODULATION

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The world is currently relying on the massive consumption of synthetic nitrogen fertilisers to maintain sufficient food production; however, this unsustainable agricultural practice is expensive and is leading to serious environmental pollution. Legume-rhizobia associated biological nitrogen fixation (BNF) via nodulation can alleviate this problem by reducing our dependence on the use of synthetic fertilisers. Therefore, a better understanding of nodule organogenesis and its regulation is important to help enhance the benefits of BNF gained from legumes. The autoregulation of nodulation (AON) process is a systemic mechanism in which rhizobia-induced CLE peptides are produced in the roots and transported to the shoots, where they induce a signal for the negative feedback of continued nodule development. Nodulation is also regulated locally by external factors, such as high soil nitrogen content. In soybeans (*Glycine Max*), three nodulation-suppressive CLE peptide encoding genes have been identified; two elicited by rhizobium inoculation (*GmRIC1* & *GmRIC2*) and one by nitrate treatment (*GmNIC1*). The rhizobium-responsive CLE genes are differentially expressed, but their overexpression results in a similar level of nodulation-inhibition. The expression of *GmNIC1* is correlated with nitrate concentration, and a 50% reduction in nodule number is achieved with constitutive expression. Each of these three nodulation-suppressing genes has a duplicate copy as a result of soybean undergoing a duplication event roughly 13 million years ago. Findings regarding the extent of expression and tissue-specific activity of each of these six genes will be presented.

POS-WED-022

ELEVATED SA SIGNALLING IN WHEAT REDUCED THE ABUNDANCE OF ARCHAEA AND NITROGEN CYCLING GENES IN THE RHIZOSPHERE IN A SOIL DEPENDENT MANNER

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Plants activate salicylic acid (SA) signalling to combat biotrophic pathogens among other functions. In the present study, we examined the effect of artificially elevated SA signalling on the microbial communities in the wheat (*Triticum* spp.) rhizosphere. We tested the hypothesis that the activation of SA signalling defence pathway alters the composition and functional diversity of rhizosphere microbial communities. Wheat was grown in two agricultural soils of different soil types (Solonetz and Calcisol), which have been used for continuous wheat cropping for many years. SA was exogenously applied to 10 day-old wheat seedlings, and rhizosphere soils were collected 72 h after SA application. Phylogenetic marker gene sequencing (16S rRNA gene) was used to characterise the bacterial and archaeal diversity. C*hitinaseA* and nitrogen (N) cycling genes including*nifH*, *arch-amoA*, *amoA*, *nosZ* and *narG* were quantified to determine the potential changes in functional diversity of the wheat rhizosphere. SA signalling marginally changed the composition of rhizosphere microbial communities in Solonetz (P=0.093) but not in Calcisol (P=0.31). In particular, SA signalling increased two *Lysobacter* and *Pseudomonas* species abundance that are involved in biocontrol in Solonetz. It also triggered a significant decrease in the archaea member *Candidatus Nitrososphaera* in Solonetz but not in Calcisol. In addition, the *amoA-arch* gene was less expressed in the rhizosphere after SA pathway activation in Solonetz, as revealed by qPCR. *nifH*, *amoA* and *nosZ* were significantly supressed at 72 h after the activation of SA signalling in Solonetz soil. Our results suggest that SA signalling alters the wheat rhizosphere microbiome and led to a decrease of bacterial components involved in N cycling in a soil dependent way.

POS-THU-023

EFFECT OF INDUCED SYSTEMIC RESISTANCE IN GRAPE BERRIES AGAINST POSTHARVEST GRAY MOULD CAUSED BY *BOTRYTIS CINEREA*

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Gray mould caused by *Botrytis cinerea*, a necrotrophic pathogen, can result in yield losses in wine grapes. The application of synthetic fungicides is an important strategy for controlling crop diseases. Intensive and exclusive use of some of the fungicides has resulted in fungicide resistance. Methyl jasmonate (MeJA), a resistance elicitor, has signalling role in eliciting induced systemic resistance (ISR) against diseases in plants. In this study, the effect of exogenous MeJA on the suppression of postharvest botrytis in green grape cultivars, 'Thomson', 'Chardonnay' and 'Vida'l and in red grape cultivars 'Merlot', 'Flame' and 'Cabernet Sauvignon' was tested. The grape bunches (15 grapes/bunch and three replicate treatments) were spray-treated with 1mM of MeJA, air dried for 3 hours. Three days after the MeJA treatment, each of the grape berry in the bunch was wounded with a needle and inoculated with 1 x 104 spores of *B. cinerea* B05.10 and incubated in the dark at 20 ºC and 85% RH. Control treatment did not receive MeJA. The lesion diameter was recorded at 7 and 14 days after inoculation. The elicitor, MeJA induced defense response by significantly suppressing the *Botrytis* gray mould in all the grape cultivars. Defense response, expressed as *PAL* gene, in grapevine berries towards *B. cinerea* , was studied. Maximum levels of induction of *PAL* gene was observed at 48 hours post inoculation and a significantly lower level of *PAL* gene was expressed in MeJA and *B. cinerea* infected grapevine berries, as compared to *B. cinerea* only infected berries.

POS-FRI-024

FUNCTIONAL ANALYSIS OF EXTRAFLORAL NECTARIES AND NECTAR OF AUSTRALIAN NATIVE WILD COTTONS

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Extrafloral (EF) nectar is the foundation of many ant-plant interactions worldwide. EF nectar is a resource that encourages ant presence, in turn resulting in protection for the plant against herbivores. There is a paucity of research on this relationship and the plant structures that underpin it in Australian species. An examination of the morphology and anatomy of EF nectaries of an Australian cotton (*Gossypium sturtianum*) revealed a suberised layer in the nectaries which indicates solutes must travel through the symplasm. $^{14}CO_2$ labelling showed the studied leaves were carbon sources and the EF nectar carbon cost is 1% of net photosynthates. Any environmental changes that affect EF nectar could have flow-on effects for ecological communities, for example rising atmospheric carbon dioxide (CO_2) levels. This study tested the response of EF nectar to elevated CO_2; the first investigation of elevated CO_2 impacts on any EF nectar system. The total volume and composition of EF nectar did not change, but there was evidence of accelerated plant development and a change in EF nectar allocation within plants. Developmental changes due to elevated CO_2 could affect the timing of EF nectar production which could have flow-on effects to ant mutualists and the defence of plants.

POS-WED-025

FOLIAR NUTRITION AND METAL ACCUMULATION IN TWO SYMPATRIC TREE SPECIES ENDEMIC TO NORTHEASTERN AUSTRALIA

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The recently described tree genus *Gossia* (Myrtaceae) comprises 48 species almost entirely restricted to the metal-enriched substrates of eastern Australia and New Caledonia. It is taxonomically delineated by an affinity for the heavy metal manganese (Mn), with some species additionally accumulating other metals; traits manifest in elevated foliar concentrations. While eastern Australian soils and climate enhance soil-Mn bioavailability that drives plant Mn toxicity in field cultivations; native plant systems are highly adapted to these inherent conditions. Mineral nutrient analyses of *Gossia* leaf material procured from herbaria, and, collected via ecological field samplings have established that this Mn-accumulating group includes several species exhibiting the rare and extreme trait of Mn hyperaccumulation. Field studies conducted to date on just a few Australian *Gossia* species have revealed unusual foliar nutritional balance and novel metal detoxification strategies; for example, whereby metabolically important mesophyll cells serve as primary sites for Mn disposal. Vacuolar Mn concentrations exceeding 0.5 M have previously been detected in leaf palisade mesophyll cells of *G. bidwillii*. This is in sharp contrast to commonly observed foliar metal detoxification strategies in hyperaccumulators of other metals, where epidermal and/or apoplastic shoot tissues serve as primary disposal sites. This ecophysiological field study evaluates foliar nutrition and metal sequestration in two sympatric *Gossia* species, *G. grayi* and*G. shepherdii*, whose global distribution is limited to North Queensland. They were previously known only from herbarium sample analyses to have elevated foliar Mn concentrations. This study employs analytical cryo-electron microscopy to examine *in vivo*foliar mineral nutrient distribution patterns in fresh leaf tissues.

POS-THU-026

INTRASPECIFIC DIFFERENCES IN OXIDATIVE STRESS SENSITIVITY OF TOMATO

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The biochemical responses of two tomato cultivars to water logging and drought stress were investigated under glasshouse conditions. We investigated the accumulation of reactive oxygen species, evidence of oxidative stress and activation of the antioxidant defence system (enzymatic antioxidants and non-enzymatic antioxidants) in leaves and roots, after 14 days of exposure to water stress. There were significant intraspecific differences in many stress responses, with lower levels of oxidative damage (hydrogen peroxide accumulation, damage to lipids, proteins and DNA) in the cultivar 'Scoresby Dwarf' under both types of water stress. Waterlogging induced hypoxia as measured by increasing ADH activity in the roots of all plants. Total carotenoid content was reduced in the pericarp of fruits of the cultivar 'Best Boy Bush' grown under water deficit and waterlogging, but not in the pericarp of 'Scoresby Dwarf' fruits. Compared to the stress-tolerant 'Scoresby Dwarf', 'Best Boy Bush' had lower activity of enzymatic antioxidants and lower production of non-enzymatic antioxidants under water stress. In conclusion, the results of this study provide strong proof of distinctive oxidative stress response patterns under the two water stress extremes with clear evidence for intraspecific differences in these responses. The findings from this study improve the understanding of biochemical changes in plants experiencing oxidative stress and can be used for the selection and development of stress tolerant tomato cultivars.

POS-FRI-027

INTERACTIONS BETWEEN DROUGHT STRESS AND CANKER PATHOGEN *(QUAMBALARIA COYRECUP)* IN MARRI *(CORYMBIA CALOPHYLLA)*: LINKING PHYSIOLOGICAL PERFORMANCE, BIOCHEMICAL DEFENCE AND DISEASE SEVERITY

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Tree mortality in many eucalypt-dominated forest ecosystems in Australia is thought to be associated with climatic extremes, and climate-driven shifts in pest and pathogen dynamics. Climatic factors are likely to predispose marri (*C. calophylla*) to the canker disease (fungal pathogen - *Q. coyrecup*), causing marri decline in Western Australia. In this study, we empirically examined the effects of drought on canker disease predisposition, and the interactions between drought stress, the pathogen and the plant physiological and biochemical (defence) response, which is yet to be investigated in any single plant pathosystem. We conducted an experiment with well-watered and drought-stressed treatments, each with inoculated and non-inoculated plants. Moreover, drought stress was applied either before inoculation, after inoculation, or throughout the experiment. Canker lesions developed in all inoculated treatments irrespective of the watering regime. However, the rate of canker growth in well-watered plants was greater in the plants that had experienced drought stress before inoculation. Photosynthesis and growth were significantly reduced due to the disease in well-watered treatments but not in drought-stressed treatments. These effects could be attributed to the reductions in stem hydraulic conductivity and stomatal conductance, rather than biochemical limitations to photosynthetic capacity. Data on secondary metabolites, potentially involved in plant biochemical defence, will also be presented for the different treatments.

POS-WED-028

CHARACTERISATION OF THE LOW AFFINITY AMMONIUM TRANSPORTERS AMF IN MAIZE

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Ammonium uptake and redistribution involves high and low affinity transport. The physiological and molecular activities of high-affinity ammonium transporters have been examined in numerous plant species. Conversely, molecular information about low-affinity ammonium transport systems is limited. Recently, a family of ammonium transport proteins called AMF1 (ammonium facilitator 1) were identified in both yeast and soybean by their genetic linkage with a membrane bound soybean transcription factor, bHLHm1. ScAMF1 and GmAMF3 homologs were shown to transport ammonium at high concentrations when expressed in yeast cells or *Xenopus laevis* oocytes. In maize two AMF1 genes, *ZmAMF1;1* and *ZmAMF1;2* were identified through sequence-based homology, and they will be characterised in this study. In contrast to ScAMF1 and GmAMF3, ZmAMF1;1 and ZmAMF1;2 failed to accumulate methyl ammonium in a yeast mutant defective in ammonium uptake. This result suggested ZmAMF1;1 and ZmAMF1;2 may have other transport activities besides that of ammonium. Currently ZmAMF1;1 and ZmAMF1;2 are being evaluated for potential roles in sugar transport in yeast mutant strains defective in sucrose and glucose uptake. In maize, the expression of both *ZmAMF1;1* and *ZmAMF1;2* were found to be upregulated by N starvation, and repressed by N resupply. A complimentary reverse genetics study is being conducted with a collection of *Mu* transposon insertions within the loci containing either *ZmAMF1;1* or *ZmAMF1;2* (B. Meeley, DuPont Pioneer). These maize lines have finished a backcrossing with the B73 and Gaspe backgrounds for three generations, and have been self-crossed and selected for homozygotic *Mu* insertions. The characterisation of these loss-of-function ZmAMF1;1 and ZmAMF1;2 lines will be presented.

POS-THU-029

BETALAIN PRODUCTION ASSOCIATED WITH ALTERED SODIUM DISTRIBUTION IN THE NEW ZEALAND ICEPLANT, *DISPHYMA AUSTRALE*

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The effects of climate change and irrigation practices have increased soil salinity levels in many parts of the world, impeding growth and reducing potential yields in many crop plants. In contrast to most crops, halophytes such as *Disphyma australe* have adaptive mechanisms that allow them to thrive in saline soil. Previous research has shown that the presence of betacyanin, a red-pigmented alkaloid, in the leaves of *D. australe* seems to correlate with increased salinity tolerance. However, the mechanism by which betacyanins might mediate salinity tolerance remains unknown. We hypothesised that betacyanins might alter sodium distribution within plant tissues, thereby avoiding the cytotoxic effects of sodium on photosynthetically active tissues. We used fluorescence microscopy and cryo-SEM coupled with energy dispersive x-ray spectroscopy on betacyanic- and green-leafed *D. australe* to explore possible associations between sodium distribution and betacyanins. The results suggest that for red leaves, sodium concentrates largely in the betacyanin-containing epidermal cells, but for the green leaves, sodium is more generally distributed. The mechanism through which betacyanins apparently divert sodium away from photosynthetic tissues warrants further investigation.

POS-FRI-030

SYNCHRONISATION OF CHANGES IN MAIZE GROWTH AND HORMONE LEVELS (ABSCISIC ACID AND ETHYLENE) DURING SOIL DRYING

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Improved understanding of plant physiological responses to drought stress in both shoot and root is important for enhancing plant drought resistance. In this study, a 6-day progressive soil drying pot experiment was conducted to investigate the synchronisation of physiological responses in maize (*Zea mays* L.) leaves and roots. During Days 2-3, when the drought was mild (23-32% soil water content in the drying pots vs. ca. 41% in the well-watered pots), root growth was stimulated. After Day 4, when the drought became severe (<18% soil water content), both root growth and leaf elongation were inhibited. The root ABA level increased gradually with the decrease in root water potential after 2-day of drying. The leaf ABA level significantly increased from Day 4, which was one day before the leaf water potential significantly decreased. The ethylene production from leaves and roots were inhibited by soil drying, which occurred later than the increase in ABA levels in leaves and roots respectively. In addition, the stomatal conductance of the younger leaves decreased from Day 3 of soil drying, which was earlier than the response of the older leaves and synchronous with the early root changes in growth rate, water potential and ABA level. Therefore, with the exception of the stomatal conductance response of the younger leaves, roots showed earlier physiological responses to soil drying than leaves. Root ABA accumulation started synchronously with the changes in root growth rate and root water potential, which suggests that ABA may be involved in regulating the stimulation of root growth under mild drought.

POS-WED-031

SEASONAL WATER RELATIONS OF FOUR CO-OCCURRING SPECIES IN A BUSHLAND FRAGMENT IN WESTERN AUSTRALIA

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Future climate change may drastically impact forest ecosystems due to increases in the dry season length and frequency/severity of drought and heat wave events. Tree mortality events, changes in vegetation structure and species distribution are likely to be associated with climate-driven extreme events. In this study, we investigated physiological responses and water relations of four species from distinct functional groups in two sites within a bushland fragment, differing in water-table depth and tree density, from Nov 2015 to May 2016. Gas exchange and water potential measurements indicate that the species (*Allocasuarina fraseriana*,*Banksia menziesii*, *Corymbia calophylla*, *Eucalyptus marginata*) exhibit distinct physiological responses during the dry season, especially where the groundwater is deeper in the soil profile. All species showed reduced photosynthesis, stomatal conductance and transpiration rates as well as declines in leaf water potentials (predawn/midday), between Dec 2015 and Feb 2016, with recovery due to rain in the following months (April and May 2016). Moreover, *B. menziesii* and *C. calophylla* maintain higher leaf water potentials, even during the dry season, whereas *A. fraseriana* and *E. marginata* showed lower values, demonstrating contrasting strategies, isohydric and anisohydric, respectively. These responses indicate that the isohydric species have a tighter control of their physiological activity when facing environmental pressures. Higher temperatures during the drier months (Dec to Feb) can increase/interact with the effects of reduced water availability and impose greater stress to the vegetation. The implications of the species strategies, higher temperatures and its impact on the physiology and water control of the study species will be discussed.

POS-THU-032

ACTIVITY-BASED PROTEIN PROFILING OF HYDROLYTIC ENZYMES INDUCED BY GIBBERELLIC ACID IN ALEURONE LAYERS ISOLATED FROM COMMERCIAL MALTING BARLEY GRAIN

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During germination, the aleurone layer of cereal grains is stimulated by gibberellic acid (GA) to secrete most of the enzymes required to degrade the carbohydrate and protein storage products of the endosperm. The complement of enzymes secreted by the aleurone layer is complex, and many specific isoforms have yet to be characterized in barley, wheat and other important cereals. Thus new approaches to study germination-related enzyme activities are required. Activity-based protein profiling (ABPP) utilizes chemical probes that bind specifically to enzymes only in their active form. An activity profile of many enzymes in a single sample can be obtained by the use of probes with distinct specificities examined under different conditions. We used different ABPP probes to determine the activity of a range of enzymes extracted from aleurone layers isolated from grains of a commercial malting barley variety incubated with or without gibberellic acid (GA). Using ABPP, enzymes found to be induced by GA were aleurain-like proteases, cathepsin-B-like proteases and a range of serine hydrolases. By using a panel of monosaccharides as potential product inhibitors, a specific active retaining β-glycosidase in the barley aleurone was identified as a putative xylanase. Our results show that ABPP can be used rapidly to identify a variety of active enzyme isoforms in cereal aleurone without the need for enzyme purification.

POS-FRI-033

GETTING TO THE ROOT OF SALT TOLERANCE IN THE MODEL CEREAL CROP, BARLEY

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Abiotic stresses are major causes of crop yield losses in agriculture significantly impacting on sustainability. Barley (*Hordeum vulgare* L.) is the most salt-tolerant cereal crop with excellent genetic/genomic resources and therefore is a good model to study salt tolerance mechanisms in cereals. Salinity results in a reduction in root growth, however, some species are able to maintain root elongation at salt concentrations that inhibit root growth; an adaptive mechanism to ensure seedling establishment and allow water and nutrient uptake. Barley cv. Clipper (malting barley) and Sahara (North African landrace 3771), the parents of the genetic mapping population used in this study, have previously been shown to have both a contrasting root growth phenotype and metabolite profile in response to the early phase of salinity stress. Clipper maintains a significantly higher relative root elongation rate in response to short-term salt stress that was associated with the synthesis and accumulation of compatible solutes indicating a potential role for these metabolic pathways in salt tolerance and the maintenance of root elongation. We now aim to identify the key genes and pathways in the roots that are involved in both perceiving osmotic changes in the soil and influencing root elongation, ultimately to increase salinity tolerance in crops. To elucidate the genetic basis for these mechanisms we are currently screening a double haploid mapping population of 146 lines derived from a Clipper x Sahara cross for their root phenotype. Future work will involve detailed genetic analysis of the mapping population using RNAseq in order to elucidate the genes involved in the maintenance of root elongation in response to salt stress. This study highlights the importance of utilizing spatial profiling and will provide us with a better understanding of abiotic stress response in plants at the tissue and cellular level.

POS-WED-034

ONLINE TOOL TO PREDICT WHEAT LEAF PHOTOSYNTHETIC TRAITS FROM HYPERSPECTRAL LEAF REFLECTANCE

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Hyperspectral leaf reflectance and partial least square regression have been used to predict Rubisco (V_{cmax}) and electron transport (J) capacity, leaf mass per unit area (LMA), chlorophyll and nitrogen content in wheat. To establish the method, data for each trait was collected from a range of wheat varieties grown and measured in both the field and greenhouse. As most wheat leaves are narrower than the optical window in the leaf clip supplied with the portable spectrometer, a mask was developed that restricts the sampling view to 11.5mm width. To facilitate the broader use of hyperspectral data to phenotype individual plants, a friendly internet-based tool was developed. In the website, it is possible to upload reflectance spectra measured with the ASD Field Spec spectrometer (350 to 2500 nm) and obtain model predictions for V_{cmax}, J, LMA and N_{area}. This tool enables anyone measuring hyperspectral leaf reflectance in wheat with this instrument to obtain parameter estimates for these traits without requiring them to have any knowledge of the partial least square regression methodology.

POS-THU-035

IDENTIFICATION OF BENEFICIAL MICROBES ON THE PHYLLOSPHERE OF *ARABIDOPSIS THALIANA* THAT INHIBIT THE SPREAD OF *ALTERNARIA BRASSICIOLA*

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The phyllosphere hosts several complex microbial communities which have the ability to tolerate harsh environmental conditions. Current literature illustrates that there could be several beneficial microbes living on the foliar surface of leaves which may provide beneficial roles for the plants health and growth. We hypotheses that beneficial phyllosphere microbes may provide resistance against several foliar pathogens. However, there has only been minimal research conducted within this area, as opposed to beneficial microbes interacting in the rhizosphere. Our first results on strawberry phyllosphere microbiomes demonstrates that we can isolate leaf bacteria that successfully inhibit fungal pathogens. In this study, we have investigated the effect of the fungal pathogen Alternaria brassicicola Isolate 7 on the leaf microbiota of Arabidopsis thaliana. Amplicons of 16S rRNA gene sequencing were conducted for bacterial and fungal identification. Along with culture inhibition assays to screen for survivors and competitors of microbial communities, additionally a collection of leaf leachates and exudates from the phyllosphere were collected to create a substrate utilisation profile and colonisation pattern of the phyllosphere microbiota. Through these methods we found that there were naturally existing microbiota on the phyllosphere. Further analyses indicated that phyllosphere microbes also inhibit the spread of foliar pathogens and this can be significantly enhanced by the production of leachates to attract beneficial microbes. Future studies will integrate this study's findings into creating new biocontrol agents, by isolating already resistant bacteria and applying them as a foliar spray to enhance plants resistance against pathogens.

POS-FRI-036

FUNCTION OF AMF PUTATIVE AMMONIUM TRANSPORT PROTEINS IN *ARABIDOPSIS THALIANA*

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A soybean transcription factor, Glycine max bHLH membrane 1 (GmbHLHm1), was previously shown to activate an uncharacterized gene in yeast, later named Saccharomyces cerevisiae ammonium facilitator 1 (ScAMF1). ScAMF1 homologs are common in plants including *Arabidopsis thaliana*. The functions of AMFs in plants are unknown. Previous experiments in yeast and *Xenopus laevis* oocytes have shown that AMFs from yeast and soybean are able to transport ammonium. To determine the function of AMFs in *Arabidopsis*, transcriptional analysis and reverse genetic approaches using T-DNA knockout lines were employed. Quantitative PCR experiments showed that three *AtAMF* genes have higher expression in older leaves than in young leaves and root tissue, with *AtAMF2* (At5g64500) having the highest expression followed by *AtAMF3* (At5g65687) and*AtAMF1* (At2g22730), respectively. Additionally, all *AtAMFs* were diurnally regulated and responded to nitrogen starvation and resupply. Growth analysis of homozygous single knockout mutants (*amf1*, *amf2* and *amf3*) grown hydroponically in nutrient solution with 1 mM NH_4NO_3 for 7 weeks showed a decrease in root dry weight but not shoot dry weight compared to wild type plants. However the photosynthetic rate, stomatal conductance and respiration rate were not significantly different between lines. Tissue localization using GUS driven by a native promoter revealed that AtAMF1 is localized in guard cells and phloem, AtAMF2 in the root tip and AtAMF3 in phloem tissue. An interesting phenotype appeared in a double knockout mutant *amf3/amf2*, which showed reduced root length when plants were grown on agar plates without the addition of sucrose. More studies need to be done to empirically characterize the function of AMF including electrophysiology, yeast complementation, and metabolomic studies.

POS-WED-037

EFFECTS OF TEMPERATURE ON SEEDLING ROOT TRAITS - TESTING A NEW HIGH-THROUGHPUT PHENOTYPING SYSTEM IN WHEAT

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Abstract: Yield progress in wheat is slowing in major production regions. Seminal root angle and root number of seedlings are considered as proxy traits for early selection of deep, rooting systems, which can allow increased water extraction in deep, drying soils during the grain filling period. Recently, a low-cost, high-throughput phenotyping method has been proposed to screen high numbers of lines (Richard *et al.* 2015. *Plant Methods* 11:13). As soil temperature varies substantially in field conditions, we tested the robustness of this method under a large range of temperatures. Seeds of 48 genotypes contrasting for seminal root angle and root number were grown in pots at constant temperatures ranging from 11 to 29 °C with non-limiting nutrient and moisture. Using a combination of image analysis and destructive measurements, seminal root angle, root number, root length and shoot length were measured. Genotype x environment interactions were observed for all the studied traits, particularly for root number. High genetic correlations between the tested temperatures were found for root angle, highlighting that genotype screening for this trait may be performed at different temperatures with little impact on genotype ranking. Thus the phenotyping method was effective across temperatures and, therefore, could be useful for screening in non-controlled environments, making it highly attractive for breeding purposes. **Keywords:** root architecture; high-throughput phenotyping; image analysis; crop improvement; *Triticum aestivum.*

POS-THU-038

VARIATIONS IN ROOT ARCHITECTURE AND MORPHOLOGY OF TWO WHEAT GENOTYPES WITH CONTRASTING PHOSPHORUS EFFICIENCY

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Root system architecture (RSA) and morphology are important for improvements of phosphorus (P) efficiency, however wheat has a sparse crown root system that is not easy to obtain without destruction for RSA analysis. In this study, a simple soil-based cultivation was developed to obtain a wheat root system without destruction for two-dimensional (2-D) RSA analysis. Two wheat genotypes RAC875 and Wyalkatchem with contrasting P efficiency were used for the studies. RAC875 produced greater grain yield than Wyalkatchem under low P but no significant difference was observed under adequate P. In contrast, RAC875 had a smaller root dry matter than Wyalkatchem at maturity. RSA traits were measured at 24 days after sowing (DAS) using Gia Roots and DIRT and root hair features were also estimated at 7 DAS to identify which root traits may be correlated to P efficiency in RAC875. P supply affected most RSA traits where P deficiency reduced the root convex hull area (CHA), root surface area, root volume, total root length, root tip number, spatial root distribution (Y). Under low P supply, RAC875 produced greater CHA than Wyalkatchem, while Wyalkatchem had significantly larger CHA than RAC875 under adequate P. Under low P, shoot dry matter was positively correlated to CHA. Wyalkatchem produced longer root hairs than RAC875. However, RAC875 had denser root hairs than Wyalkatchem under low P but no significant differences occurred at adequate P. Larger CHA and denser root hairs appears to support a greater biomass and yield production in RAC875 under P deficiency. Thus, convex hull area and root hair density are potential indicators when screening for P efficient wheat.

POS-FRI-039

PHYSIOLOGICAL INTRA-SPECIFIC VARIATION TO ELEVATED CO_2 AND WATER LIMITATION IN *EUCALYPTUS GRANDIS* - WHERE DO "WINNERS" COME FROM?

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Eucalyptus is a highly diverse tree genus and selected species are the most sought after hardwood plantation species worldwide. Each of these ecologically as well as commercially important species have a wide ranging natural habitat and large associated intra-specific genetic diversity. However, the plasticity of their genetic diversity to respond to future climates is largely unknown. Yet, this information is crucial in forward planning of ecosystem management and financial gain. Our research is addressing the role of genotypic (*G*) diversity in provenances of *E. grandis* to adjusting to their environment (*E*). Particularly, the interaction *G* x *E*, represented by elevated concentrations of atmospheric CO_2 (eCO_2) and water availability is assessed. We found that trees originating from colder climates produced larger biomass than trees coming from warmer climates when supplied with sufficient water. Furthermore, even though photosynthetic rates (*A*) increased in all provenances in eCO_2 under well-watered conditions, trees from colder climates exhibited greater plasticity when water was limited. They showed greater increase in *A* under eCO_2compared to rates of *A* under ambient CO_2. Our research highlights the importance of exploiting intra-specific variation in*Eucalyptus* to optimize tree growth under forecasted climatic conditions. Our results demonstrate that provenances of *E. grandis*originating from colder climates appear to be more suited in coping with future climate challenges like eCO_2 and water limitation.

POS-WED-040

ASSESSING CORRELATIONS BETWEEN LEAF AND ROOT TRAITS OF PRUNUS ROOTSTOCKS UNDER WATER DEFICIT AND SALINITY STRESS

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Recent interest in trait-based plant ecology has sparked the search for a root economics spectrum (RES) and the evidence of RES mirroring the leaf economics spectrum. However, studies exploring these correlations under abiotic stress, and the differential magnitude of correlations among congeneric plant taxa, are limited. By imposing water and salinity stresses on seven *Prunus* rootstock varieties, this study aimed to test the hypothesis that fine-root traits would match leaf traits, which are typically coordinated along an axis from resource acquisitive to resource conservative traits. Seedling plants of seven *Prunus* rootstock varieties were randomly allocated into three water and salinity stress treatments for two months in a glasshouse. Plants in the well-watered treatment were irrigated to the point of run-off daily. The severe drought treatment was imposed by adding 33% of evapotranspiration of well-watered plants daily. Plants in the salinity treatment were supplied with mixed chloride solution (with electrical conductivity 3.3 $dS\ m^{-1}$) to the point of run-off daily. Besides plant biomass, measurements included key above- and below-ground plant traits (leaf mass area, root density, root diameter, root length, root surface area), stem water potential and leaf gas exchange. The seven *Prunus* rootstocks differed in the extent of growth responses to stresses, with the effects of severe drought generally being larger than those of salinity stress. Meanwhile, their differential sensitivity to stress is correlated between above- and below-ground plant traits. The findings showed important correlations between above- and below-ground plant traits under abiotic stress. This study contributes to knowledge of differential responses of plant traits between congeneric plant taxa under abiotic stress, useful for plant physiologists and modellers.

POS-THU-041

SNAI1 EXPRESSION IS UP-REGULATED IN COLORECTAL CANCER AND CORRELATES WITH INTESTINAL STEM CELL MARKERS

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SNAIL family of transcriptional regulators (SNAI1, SNAI2 and SNAI3) are well known for their role in mediating epithelial-mesenchymal transitions (EMTs), which is fundamental for the acquisition of tumour invasiveness (Hay 1995). Members of the Snail family have been shown to be upregulated in several human cancers and are frequently associated with poor prognosis (Peinado et al. 2007). However, not much is known about their role in regulating epithelial cancer stem cells. Our previous study suggests that up-regulation of Snai1 in the normal mouse small intestinal epithelium results in an increase in intestinal stem cell (ISC) number and epithelial cell proliferation (Horvay et al 2015). The aim of this project is to investigate the role of the SNAIL proteins in colorectal cancer and whether these molecules regulate cancer stem cells. Expression levels of SNAIL family members and ISC markers were analysed in tumour and matched normal tissues collected from 69 colorectal cancer patients by Droplet Digital PCR (ddPCR) and were compared with clinical data. Results from this study suggest that SNAI1 is upregulated in colorectal cancer and expression of SNAIL family members correlates with expression of intestinal stem cell markers in these patients. Moreover, expression of SNAI1 has been found to correlate with tumour stage in this patient cohort. We are also examining the functional role of Snai1 in mouse models of intestinal polyp formation. References Hay ED (1995). An overview of epithelio-mesenchymal transformation. Acta Anat 154: 8–20. Peinado H, Olmeda D, Cano A (2007). Snail, Zeb and bHLH factors in tumour progression: an alliance against the epithelial phenotype? Nat Rev Cancer 7: 415–28. Horvay K, Thierry J, Casagranda F, Perreau VM, Haigh K, Nefzger CM et al. (2015) Snai1 regulates cell lineage allocation and stem cell maintenance in the mouse intestinal epithelium. EMBO J 34(10): 1319-35.

POS-FRI-042

CHARACTERISATION OF THE ROLE OF *POLYCYSTIN-1* DURING EMBRYONIC LYMPHANGIOGENESIS

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The lymphatic system is essential for maintaining homeostasis and immunity. Abnormal development of lymphatic vessels can lead to lymphedema, organ and structure impairment, and immune deficiencies. Knowledge of lymphangiogenesis is valuable for tackling these diseases, but little is known about the molecular regulators of lymphatic network formation, control of branching morphogenesis and cell polarity. We have found that *Polycystin-1* knockout in mice leads to defective morphogenesis of developing lymphatic networks and altered polarity of lymphangiogenic sprouts. Polycytin-1 can modulate planar cell polarity pathways and interestingly, we find that mutants in other components of these pathways (Ryk, Wnt5a) show very similar lymphatic defects. Moreover, *Polycystin-1* genetically interacts with Wnt5A in lymphangiogenesis. In *Polycystin-1* and *Wnt5A* double knockout mice, the lymphatic network of the dorsal embryonic skin has reduced branch points, loops and migration towards the midline compared with WT and *Polycystin-1* knockout mice. Our data show that Polycystin-1 is also required for recruiting core planar cell polarity components. Overall, this study suggests that Polycystin-1 is a novel partner of non-canonical Wnt, planar cell polarity signalling controlling network morphogenesis during lymphangiogenesis.

POS-WED-043

TOB1 AND TOB2 MARK DISTINCT RNA PROCESSING GRANULES IN DIFFERENTIATING LENS FIBRE CELLS

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Lens fibre cell differentiation involves a complex interplay of growth factor signals and tight control of gene expression via transcriptional and post-transcriptional regulators. Recent studies have demonstrated an important role for RNA-binding proteins, functioning in ribonucleoprotein granules, in regulating post-transcriptional expression during lens development. Here we have documented the expression of Tob1 and Tob2, members of the BTG/Tob family of RNA-binding proteins, in the developing lens. Both Tob1 and Tob2 mRNA were detected by RT-PCR in embryonic and postnatal lenses and were present in both epithelial and fibre cells in postnatal lenses. By in situ hybridisation, Tob1 and Tob2 mRNA were most intensely expressed in the early differentiating fibres, with weaker expression in the anterior epithelial cells and were downregulated in the germinative zone of E15.5 lenses. Tob1 protein was detected from E11.5 to E16.5 and was predominantly detected in large cytoplasmic puncta in early differentiating fibre cells, often colocalising with the P-granule marker, Dcp2. Occasional nuclear puncta were also observed. By contrast, Tob2 was detected in later differentiating fibre cells in the inner cortex and did not co-localise with Dcp2. The identity of these Tob2+ granules, which often appear as a series of interconnected peri-nuclear granules, is currently unknown. In vitro experiments using rat lens epithelial explants treated with or without a fibre differentiating dose of FGF2 showed that both Tob1 and Tob2 are up-regulated during FGF-induced differentiation. In differentiating explants, Tob1 also co-localised with Dcp2 in large cytoplasmic granules. These findings suggest that Tob proteins play important, but distinct, roles in RNA processing during lens fibre differentiation.

POS-THU-044

ORIGIN OF VASCULAR PROGENITORS DRIVING MELANOMA ANGIOGENESIS *IN VIVO*

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The development of new blood and/or lymphatic vessels is a pre-requisite for Melanoma growth and spread through metastasis. With the view to better identify and characterize the source of de novo endothelium in cancer, we utilised flow cytometry and immunofluorescence to characterise endothelial populations in B16 melanoma. We identify endothelial cells derived from resident vascular beds and not from hematopoietic lineages. Among Lin-CD34+ cells, expression levels of VEGFR2 and CD31 defined three distinct endothelial sub-populations. Furthermore, using lineage tracing of VE_cadherin expressing endothelial cells (Cdh5-Cre/ER RosaYFP), we demonstrate a maturation sequence from progenitor (P) via transit amplifying (TA) to fully differentiated (D) cells in B16 melanoma as well as in skin wounds. Sox18, a transcription factor essential in vascular development was then considered as a functional marker of the progenitor population. Sox18 expression is lost in the adult, with reexpression reported in endothelial cells only in pathogenic situations of angiogenesis. We utilized Sox18CreER/Rosa-YFP reporter mice and showed that progenitors activate Sox18. Fate tracking over multiple tumour time-points revealed that by day 5 after melanoma cell injection, only progenitors have been recruited to the tumour; by day 10, there is a sequential progression to fully differentiated cells. Immunohistochemistry for vascular markers on these tumours demonstrate that progenitor cells originate from venous vascular beds and can give rise to de novo arterial, veinous and lymphatic structures in the tumour. This highlights the usefulness of strategies targeting vascular progenitors in cancer.

POS-FRI-045

TRANSCRIPTIONAL REGULATION OF NFIX BY NFIA/B DRIVES LATE GLIOGENESIS IN THE DEVELOPING SPINAL CORD

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During mouse spinal cord development ventricular zone progenitor cells transition from producing neurons to producing glia at approximately embryonic day 11.5, a process known as the gliogenic switch. The transcription factors Nuclear factor I (NFI) A and B orchestrate this developmental transition, but the contribution of a third NFI member, NFIX, remains unknown. Here we reveal that NFIX is expressed by ventricular zone progenitor cells within the spinal cord after the onset of NFIA and NFIB expression, and after the gliogenic switch has occurred. Mice lacking NFIX exhibit normal neurogenesis within the spinal cord but aspects of terminal glial differentiation are impaired. Finally, we demonstrate that NFIA and NFIB can each transcriptionally activate NFIX expression. These data highlight the importance of the NFI family in regulating gliogenesis within the embryonic spinal cord, and reveal that NFIX is part of the downstream transcriptional program through which NFIA and NFIB promote gliogenesis in ventricular zone progenitor cells.

POS-WED-046

Usp9x-DEFICIENCY DISRUPTS THE MORPHOLOGICAL DEVELOPMENT OF THE POSTNATAL HIPPOCAMPAL DENTATE GYRUS

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Within the adult mammalian brain, neurogenesis persists within two main discrete locations, the subventricular zone lining the lateral ventricles, and the hippocampal dentate gyrus. Neurogenesis within the adult dentate gyrus contributes to learning and memory, and deficiencies in neurogenesis have been linked to cognitive decline. Neural stem cells within the adult dentate gyrus reside within the subgranular zone (SGZ), and proteins intrinsic to stem cells, and factors within the niche microenvironment, are critical determinants for development and maintenance of this structure. Our understanding of the repertoire of these factors, however, remains limited. The deubiquitylating enzyme USP9X has recently emerged as a mediator of neural stem cell identity. Furthermore, mice lacking *Usp9x* exhibit a striking reduction in the overall size of the adult dentate gyrus. Here we reveal that the development of the postnatal SGZ is abnormal in mice lacking *Usp9x*. *Usp9x* conditional knockout mice exhibit a smaller hippocampus and shortened dentate gyrus blades from as early as P7. Moreover, the analysis of cellular populations within the dentate gyrus revealed reduced stem cell, neuroblast and neuronal numbers and abnormal neuroblast morphology. Collectively, these findings highlight the critical role played by USP9X in the normal morphological development of the postnatal dentate gyrus.

POS-THU-047

PROTEOGLYCANS IN HUMAN MESENCHYMAL STEM CELL NEUROGENESIS

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Human mesenchymal stem cells (hMSCs) self-renew and possess multi-lineage differentiation potential, including the neural lineages (neurons, astrocytes, and oligodendrocytes). Cell lineage differentiation potential is often influenced by the localised microenvironment or niche, in which the extracellular matrix (ECM) is a major component. Proteoglycans (PGs) are major constituents of the neural ECM and are characteristically comprised of a core protein to which a series of glycosaminoglycan (GAG) side chains attach at specific sites. Recent findings by our group have identified specific PGs as potential novel markers of human neural stem cell (hNSC) lineage specification by demonstrating their role in hNSC maintenance and lineage commitment. In addition, our group has identified a potential role for HSPG core proteins, syndecans and glypicans, in hMSC neural lineage differentiation. hMSC populations (n = 3) were differentiated under neural lineage culture conditions through direct terminal differentiation and terminal differentiation via intermediate sphere formation. RNA and protein were collected throughout differentiation at days 7, 14, and 28 during basal lineage differentiation conditions along with cultures augmented for stimulation (heparin) and inhibition (sodium chlorate) of PG GAG side chains. Gene expression analysis showed distinct differences in PG expressions between neural specific culture conditions, stages of differentiation, and between untreated, heparin and chlorate-treated cultures. Overall, the data strongly suggests PGs, particularly syndecans and glypicans may be key players in hMSC neurogenesis and we aim to examine these PGs in further detail. A deeper understanding of the complex and dynamic processes mediating hMSC neurogenesis will likely enable advances in stem cell therapy for application to the understanding and repair of neurological disorders.

POS-FRI-048

CAN MCHERRY OR ZSGREEN1 FUSION PROTEINS AFFECT NULLBASIC'S ANTIVIRAL ACTIVITY IN CD4+ CELLS?

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Human immunodeficiency virus-1 (HIV-1), the causative agent of autoimmune deficiency syndrome (AIDS), is a major health problem world-wide resulting in approximately 5000 new infection cases daily. Because of the large number of fatalities consequent of HIV, the importance of treatment research is vital. As of now, HIV-1 infections are being effectively controlled by antiretroviral therapy (ART) which inhibits virus replication in infected individuals to undetectable levels. However, suspension of ART leads to a rebound in viral load. Nullbasic, a mutant of HIV-1 transactivator of transcription(Tat) protein, exhibits strong antiviral activity in lymphocytes through the inhibition of multiple steps of HIV-1 replication cycle. Recently, it was noted that the antiviral activity of Nullbasic in CD4+ primary cells varied following its fusion with fluorescent proteins mCherry and ZsGreen1. Nullbasic-mCherry resulted in a reduction of antiviral activity, a contrast seen with Nullbasic-ZsGreen1. In this study, we investigated if this was a result of either the conformational changes in Nullbasic imparted by the respective protein partners or the multimeric states of each fluorescent protein. To achieve this, a self-cleaving peptide T2A was used to allow Nullbasic to exist independently compared Nullbasic-mCherry/ZsGreen1. Additionally, dimeric and tetrameric forms of Nullbasic were obtained by the addition of synthesized GCN4 leucine zippers to Nullbasic and the antiviral activity was compared to its original monomeric form of Nullbasic. All of the constructed Nullbasic expression vectors were transduced into Jurkat cells and human CD4+ primary cells. Positive cells were selected by a cell sorter and infected with HIV-1. HIV-1 levels were measured by p24ELISA.

POS-WED-049

TOWARDS THE EXPRESSION OF A CELLULOSE SYNTHASE-LIKE F (CSLF) PROTEIN, RESPONSIBLE FOR THE SYNTHESIS OF MIXED LINKAGE β-GLUCAN, IN A MICROBIAL MODEL SYSTEM

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(1,3)(1,4)-β-glucans, also known as mixed linkage glucans (MLGs), are major cell wall matrix polysaccharides in grasses with a distinct structure comprising both β-(1,3)- and β-(1,4)-glucosidic linkages. This unique structure makes MLG an important source of soluble dietary fibre conferring benefits to human health. The cellulose synthase-like F (CSLF) glycosyltransferases, of the CAZy GT2 family, are primarily responsible for the synthesis of MLG, with the major isoform CSLF6 localised and integral to the plasma membrane. However, despite the importance of MLG to both plant growth and human health, the molecular mechanism by which this polysaccharide is synthesised is not well understood. With the recent publication of the first crystal structures for a GT2 family member, a bacterial cellulose synthase (BcsA), new opportunities are created to draw parallels between related proteins which synthesise diverse polysaccharide products. The optimisation of an *Escherichia coli* expression host for the structural characterisation of CSLF6 would provide a powerful tool for exploring differences between GT2 proteins. We will present our latest findings directed towards the expression and isolation of catalytically active CSLF6 in *E.coli*.

POS-THU-050

THE SOLUTION STRUCTURE OF SR33 CHALLENGES PARADIGMS FOR COILED-COIL DOMAIN DIMERIZATION IN PLANT NLR IMMUNITY RECEPTORS

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Plants utilize intracellular immunity receptors, known as NLRs (nucleotide-binding oligomerization domain-like receptors) to recognize specific pathogen effector proteins and induce immune responses. These proteins provide resistance to many of the world's most destructive plant pathogens, yet we have a limited understanding of the molecular mechanisms that lead to defense signaling. We examined the wheat NLR protein Sr33, which is responsible for strain-specific resistance to the wheat stem-rust pathogen, *Puccinia graminis f. sp. tritici*. We present the solution NMR structure of a coiled-coil fragment from Sr33, which adopts a four-helix bundle conformation. Unexpectedly, this structure differs from the published dimeric crystal structure of the equivalent region from the orthologous barley powdery mildew resistance protein, MLA10, but is similar to the structure of the distantly related potato NLR protein, Rx. We demonstrate via SEC-MALS and SAXS that these regions are in fact largely monomeric and adopt similar folds in solution in all three proteins, suggesting that the CC domains from plant NLRs adopt a conserved fold. However, larger C-terminal fragments of Sr33 and MLA10 can self-associate both *in vitro* and *in planta* and this self-association correlates with their cell death signaling activity. We show that the minimal region of the CC domain required for both *in planta* cell death signaling and self-association extends to amino acid 142, thus including 22 residues absent from previous biochemical and structural protein studies. These data suggest that self-association of the minimal CC domain is necessary for signaling but that this is likely to involve a different structural basis than previously suggested.

POS-FRI-051

POTENT NEUROPROTECTION AFTER STROKE AFFORDED BY A DOUBLE-KNOT SPIDER TOXIN THAT INHIBITS ACID-SENSING ION CHANNEL 1A

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Acid-sensing ion channels (ASICs) are pH-dependent cation channels that are widely expressed throughout the nervous system. ASIC1a is the dominant ASIC subtype in mammalian brain and it is a key contributor to the neurodegeneration observed following ischaemic stroke. We discovered a novel spider-venom peptide (Hi1a) that blocks ASIC1a with subnanomolar potency and is 30-fold more selective for ASIC1a over other ASIC subtypes. Determination of the structure of Hi1a using NMR revealed that is a double knot toxin comprised of two inhibitor cystine knot motifs joined by short linker. Hi1a has a unique mode of action that differs from previously characterised ASIC1a modulators. Inhibition of ASIC1a by Hi1a is not complete as ~30% of channel current remains after exposure to saturating concentrations of peptide. Moreover, the inhibition of ASIC1a pH-induced currents is irreversible. We evaluated whether Hi1a is neuroprotective in a rat model of transient focal ischaemia. A single low dose of Hi1a (2 ng/kg, administered to the cerebroventricular) up to 8 hours post-stroke markedly reduced infarct size and neurological deficits without adverse effects on motor activity. Thus, Hi1a represents an exciting lead molecule for development of therapeutics for neurodegeneration and other pathophysiological conditions involving ASIC1a.

POS-WED-052

POTENT, BROAD-SPECTRUM SERINE PROTEASE INHIBITION ACHIEVED BY A SIMPLIFIED BETA-SHEET MOTIF

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Engagement of an extended beta-hairpin is a common substrate/inhibitor interaction at the active site of serine proteases and is a key feature of Laskowski mechanism inhibitors that present a substrate-like loop to a target protease. This loop is cleaved but subsequently religated forming a stable protease/inhibitor complex. Laskowski inhibitors are ubiquitous in nature and are used extensively in serine protease inhibitor design. However, most studies concentrate on engineering sidechain interactions rather than the direct contributions of the substrate-like beta-sheet to enzyme inhibition. Here we report the structure of an optimised beta-sheet inhibitory motif within the Sunflower Trypsin Inhibitor (SFTI) and describe its interactions in a bovine trypsin/SFTI crystal structure. For the first time we demonstrate the ability of a crystalised protease to cleave and religate a Laskowski inhibitor and show that an optimised SFTI variant, SFTI-TCTR-$N_{12}N_{14}$ has an internal hydrogen bond network that engages the inhibitor sidechains that would normally interact with a target protease, making main-chain interactions the dominant feature driving complex formation. Despite having reduced sidechain interactions, this SFTI variant is remarkably potent, inhibiting trypsin with a K_i of 0.7 nM. Furthermore, it inhibits a chymotrypsin-like enzyme, kallikrein-related peptidase 7 with a K_i of 16.4 nM compared to wild type SFTI with a K_i of over 10000 nM. Molecular modelling of the SFTI/KLK7 complex again indicates an interface dominated by bata-sheet interactions, highlighting the importance of this motif and the adaptability of SFTI as a scaffold for inhibitor design.

POS-THU-053

DEVELOPMENT OF *OV*-GRN-1 FRAGMENT AS A WOUND HEALING AGENT

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Granulins are a family of protein-based growth factors that are involved in a wide range of physiological functions and disease processes from inflammation to tumour growth. The liver-fluke granulin, *Ov*-GRN-1, isolated from a carcinogenic liver fluke *Opisthorchis viverrine*, has been recently shown to induce angiogenesis and significantly accelerate wound repair *in vivo* and *in vitro*. Therefore, *Ov*-GRN-1 offers potential as a novel biologic tool for treating acute and chronic wounds where the normal tissue repair mechanisms are overwhelmed, such as diabetic ulcers. We are studying the structure-function relationships of *Ov*-GRN-1 with the aim of engineering more stable, potent that are cheaper to manufacture while the bioactivity still maintained. To determine the minimal optimized fragment of *Ov*-GRN-1, analogues were synthesised using solid phase peptide synthesis (with FMOC chemistry. The peptides were purified with RP-HPLC and characterised using mass spectrometry. Structural analysis was done using NMR spectroscopy and biological activity examined using real-time cell proliferation monitoring using an xCELLigence unit and *in vitro*Scratch wound healing assay. The most active peptides in the cell-based assays were tested in an *in vivo* wound-healing assay. The data showed that it is feasible to minimise the *Ov*-GRN-1 protein and maintain bioactivity.

POS-FRI-054

INTERACTIONS OF PHOSPHOINOSITIDES WITH NEURONAL MUNC18A/SYNTAXIN1A IN VESICLE TRAFFICKING

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The Sec1/Munc18 (SM) proteins and the soluble N-ethymaleimide-sensitive factor attachment protein receptors (SNAREs) play an essential role in vesicle docking and fusion. The SNARE complex is composed of the target membrane SNAREs (t-SNAREs, Sx1a and SNAP25 in neurons) and the vesicle membrane SNARE (v-SNARE, VAMP2). The formation of the SNARE complex leads to membrane fusion. Neuronal SM protein Munc18a is essential for neurotransmission release and binds specifically to its cognate Sx1a. Structural studies have revealed the two binding modes of the interaction between Munc18a and Sx1a (N-terminal peptide and “closed”) (1). The Munc18a/Sx1a crystal structure showed that Munc18a binds to Sx1a in a closed conformation that is incompatible with SNARE complex formation (2, 3). We previously solved the crystal structure of Munc18a in complex with a non-cognate N-peptide from syntaxin4 (4). Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) was shown to interact with the polybasic residues of Sx1a (260KARRKK265) adjacent to the transmembrane domain (5, 6). The computational analysis has suggested that PI(4,5)P2 lipid regulates the phosphorylation of syntaxin N□terminus (7). By using lipid binding assays, we show that both Munc18a and Sx1a bind strongly to PI(3,5)P2, PI(4,5)P2 and also monophosphoinositides. References 1. Archbold JK, Whitten AE, Hu S-H, Collins BM and Martin JL (2014) Curr Opin Struct Biol 29, 44-51 2. Misura K, Scheller R, Weis W. (2000) Nature 404, 355-62. 3. Burkardt P, Hattendorf DA, Weis WI and Fasshauer D (2008) EMBO J 27, 923-933. 4. Hu S.-H., Christie MP, Saez NJ, Latham CF, Jarrott R, Lua LH, Collins BM and Martin JL (2011) PNAS 108, 1040-1045. 5. Paolo, G.D. & Camilli, P.D. (2006) Nature 443, 651-657 6. van den Bogaart G, Meyenberg K, Risselada HJ, Amin H, Willig KI, Hubrich BE, Dier M, Hell SW, Grubmüller H, Diederichsen U, Jahn R. (2011) Nature 479, 552-555 7. Khelashvili G., Aurelio Galli A. and Weinstein H. (2012) Biochemistry 51, 7685-7698.

POS-WED-055

NEW CRYSTAL STRUCTURES OF THE CATALYTIC SUBUNIT OF *ARABIDOPSIS THALIANA* ACETOHYDROXYACID SYNTHASE REVEAL NEW INSIGHTS IN THE MECHANISM OF INHIBITION OF COMERCIAL HERBICIDES

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Acetohydroxyacid synthase (AHAS, E.C. 2.2.1.6) is the first enzyme in the branched-chain amino acid biosynthesis pathway. Five of the most widely used commercial herbicides (*i.e.* sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidinyl-benzoates, and sulfonylamino-cabonyl-triazolinones) target this enzyme. The catalytic subunit of *Arabidopsis thaliana* (*At*) AHAS has previously been co-crystalized with sulfonylureas and imidazolinones. The structures showed these two classes of herbicides bind to the enzyme by significantly different mechanisms of induced fit. This raises questions as to how the other three classes of commercial herbicides associate with the enzyme, and what changes occur in the uninhibited enzyme in order to bind the herbicides. Here, we have determined the crystal structures of *At*AHAS in the absence of inhibitor (2.9 Å resolution), and in complex with two pyrimidinyl-benzoates and two sulfonylamino-carbonyl-triazolinones (2.7-2.8 Å resolution). These new structures show that the herbicide-binding site in uninhibited *At*AHAS is already highly ordered and folded. This is in contrast with unliganded*Saccharomyces cerevisiae* AHAS, which shows that the critical regions for herbicide binding are disorded. In addition, these herbicides cause a side reaction that leads to the breakdown of the cofactor ThDP. The structures provide an unprecedented understanding of the mechanism of inhibition of plant AHAS by the commercial herbicides and new insights into the molecular basis of weed resistance that results from application of these herbicides. These data then can be exploited to design more sophisticated AHAS inhibitors as advanced herbicides.

POS-THU-056

6-OXOPURINE PHOSPHORIBOSYLTRANSFERASE AS AN ANTIMALARIAL DRUG TARGET

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6-Oxopurine phosphoribosyltransferase catalyzes the formation of the 6-oxopurine nucleoside monophosphates from a purine base and 5′-phospho-α-D-ribosyl-1-pyrophosphate. A divalent metal ion is required for the reaction to proceed. This enzyme has been identified as an important target for antimalarial chemotherapy because it is their only pathway for the synthesis of the purine nucleoside monophosphates required for DNA/RNA production. Thus, inhibition of this enzyme should result in killing of the malarial parasite [1]. It has previously been shown that acyclic nucleoside phosphonates (ANPs) have been successfully developed as antiviral agents by targeting DNA polymerases and reverse transcriptases [2]. We have used rational structure-based drug design, including X-ray crystal structures, to propose new ANPs that we have subsequently synthesized. These new compounds are excellent inhibitors of Plasmodium falciparum HGXPRT, with Ki values as low as 0.08 and 0.01 μM for Plasmodium vivax HGPRT [3-5]. We have also developed prodrugs of these ANPs that exhibit antimalarial activity against Pf lines with IC50 values as low as 0.8 μM. These compounds have low cytotoxicity against human cells [4, 6]. [1] de Jersey et al., & Guddat, Curr Top Med Chem, 11 (2011) 2085. [2] De Clercq, Holý, Nat Rev Drug Discov, 4 (2005) 928. [3] Keough et al., & Guddat, J Med Chem, 52 (2009) 4391. [4] Keough et al, & Guddat, J Med Chem, 58 (2015) 827. [5] Keough et al., & Guddat, J Med Chem, 56 (2013) 2513-2526. [6] Hockova, et al., & Guddat, Bioorg Med Chem, 23 (2015) 5502.

POS-FRI-057

RECONSTRUCTION OF A THERMOSTABLE CYTOCHROME P450 OMEGA-FATTY ACID HYDROXYLASE

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Ancestral reconstruction is a growing field of bioinformatics, involving the inference of an ancestral protein based on the available extant sequences of its descendants. This approach has been used previously to generate proteins significantly more thermostable than the extant forms, including cytochrome P450 enzymes belonging to the CYP3 and CYP2 families. Presented here is the use of a maximum likelihood, joint reconstruction approach to generate the ancestor of the CYP4ABXZ clade containing the CYP4A, CYP4B, CYP4X and CYP4Z subfamilies. These proteins consist primarily of fatty acid omega-hydroxylases with potential applications in the production of biopolymers, but currently lack the thermostability required of industrial biocatalysts. CYP4ABXZ was successfully synthesised and expressed in *E. coli*, along with corresponding extant forms (human CYP4A11 and rabbit CYP4B1), and was shown to demonstrate a significant increase of >25°C in thermostability over the extant forms, as measured by $^{10}T_{50}$ (the temperature at which half the protein remained intact after ten minutes). CYP4ABXZ will provide a robust scaffold for the future engineering of thermostable omega-hydroxylases for industrial applications.

POS-WED-058

STRUCTURAL CHARACTERISATION OF TRIMERIC TOXINS FROM AUSTRALIAN SNAKE VENOMS

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Cytotoxic phospholipases A_2 (PLA_2) are a common feature of Australian snake venoms. Some of these proteins form heterogeneous trimers, which are composed of α (primary), β and γ (ancillary) subunits. The paucity of structural data for these toxins has limited investigations into the nature of structure-function relationships and cytotoxic activities. In the current work, nanoelectrospray ionisation mass spectrometry (nanoESI-MS) and ESI-ion mobility MS (IM-MS) were used to compare PLA_2 from the venoms of *Acanthophis antarcticus*, *Oxyuranus microlepidotus*, *Oxyuranus scutellatus* and *Tropidechis carinatus*. This included confirmation of subunit composition, stoichiometries and cross-sectional areas. These results confirmed the presence of trimers (αβγ) with mass differences between isoforms arising from variations in the glycosylation of the γ subunit species. When collisional energies were applied the trimers dissociated into two species: β monomers and αγ assemblies. A comparison of these dissociation products to the subunits free in solution indicated that only the α and γ subunits lost charges (six positive) when assembled. This suggests that the β monomers are held in place predominantly by hydrophobic interactions, while the α and γ subunits are bound by electrostatic interactions. Finally, the data from IM-MS experiments suggest that these toxins have similar cross-sectional areas. This study points to structural similarities between trimeric PLA_2 from Australian snakes and this information will be used as a basis to investigate the structure-function relationships of these toxins. Crystallography trials are in progress to confirm these observations.

POS-THU-059

STRUCTURAL CHARACTERISATION OF EUKARYOTIC CAD COMPLEX

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Carbamoyl phosphate synthetase II (CPS II), aspartate transcarbamoylase (ATC), and dihydroorotase (DHO) are three enzymes involved in the first three steps of de novo pyrimidine biosynthesis. In bacteria, these proteins are present in individual enzymes and function independently; however, in eukaryotes they are often found in a covalently linked form. In mammals, the enzymes are present as a large, multienzyme complex called CAD (CPS II-ATC-DHO, 243 kDa) and this complex has been proposed to form a homo-hexamer of ~1.5 MDa in the cellular environment. It is known that over-expression of CAD is required for proliferation and is associated with tumour cell development; thus, understanding the structural basis of the complex will be useful in cancer biology. CAD in yeast, called URA2, is a bifunctional protein that has CPS II and ATC activity only. The DHO domain is substituted with pseudo-DHO amino acids sequence, which has lost its enzyme activity. The separately translated active DHO (URA4) compensates this loss of function, and this is homologous to bacterial type II DHO. This suggests the sequential gene duplication and fusion events occurred for CAD; thus, studying yeast URA2 may help understanding the molecular evolution. Crystal structure of the pseudo-DHO domain was determined at high resolution (1.2 Å) and biochemical studies such as enzyme assay, MALS, and SAXS were performed. The protein presents in equilibrium between dimer and hexamer in solution, and this suggests its potential role of the domain in hexamerisation of the CAD complex in cellular environment despite its inactivity.

POS-FRI-060

STRUCTURAL STUDIES OF THE INTERACTION OF GATING MODIFIER TOXINS WITH VOLTAGE-GATED SODIUM CHANNELS

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The human voltage-gated sodium channel $Na_V1.7$ plays a vital role in the amplification of pain signals in sensory neurons. While gain-of-function mutations in the SCN9A gene, which encodes the pore-forming α-subunit of $Na_V1.7$, cause erythermalgia and paroxysmal extreme disorder, loss-of-function mutations in the same gene lead to a congenital indifference to pain. Thus, $hNa_V1.7$ is a promising analgesic target. Numerous spider-venom peptides inhibit $hNa_V1.7$ by binding to the domain II voltage-sensor domain and inhibiting channel activation. We aim to develop a molecular understanding of the interactions between these peptides and $hNa_V1.7$ with a view towards rational design of peptide-based analgesics that selectively this channel. In attempt to obviate the difficulties in producing stable $hNa_V1.7$ for structural studies, we produced a chimeric Na_V channel in which the peptide-binding regions of the domain II voltage sensor of $Na_V1.7$ were transplanted into a bacterial sodium channel, Na_VRh, which has previously been successfully crystallised. The chimeric Na_V channel was successfully expressed and purified, and shown to bind peptides that normally target $hNa_V1.7$.

POS-WED-061

ENGINEERING A C-MANNOSYLATION PATHWAY INTO *PICHIA PASTORIS*

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Producing recombinant proteins on an industrial scale with human-like patterns of glycosylation remains as an important challenge in biotechnology. Heterologous expression of proteins in mammalian cells is expensive relative to other expression systems and is complicated by its susceptibility to viral contamination. Yeast protein production systems are key to delivering low-cost biologics because they are inexpensive to culture but maintain the benefits of a eukaryotic expression host. However, yeast glycosylation pathways are significantly different to those in humans, which limits its usefulness for manufacturing biologics. This problem has driven significant efforts to 'humanise' glycosylation pathways in yeast. While efforts have mainly focused on producing proteins with homogenous human-like N-glycans, engineering other mammalian glycosylation pathways into yeast remains an important goal. Tryptophan C-mannosylation is an unusual co-translational modification found in metazoans. This modification occurs on the WXXW consensus motif in thrombospondin repeats (TSRs) and type-I cytokine receptors, as well as some cytokines, hyaluronidases, RNAses, and virus glycoproteins. Establishing yeast strains capable of Trp-C-mannosylation would facilitate the low cost production of proteins that could be useful as biologics or vaccines. Yeast possesses no detectable C-mannosyltransferase activity, but they do produce a large quantity of mannose phosphate dolichol, the substrate utilized by C-mannosyltransferases. We engineered *Pichia pastoris* to constitutively express the C.*elegans* C-mannosyltransferase localised to the ER. This heterologous enzyme harnessed intracellular mannose phosphate dolichol to drive C-mannosylation of proteins over-expressed in this system. This refinement in the 'humanisation' of yeast glycosylation pathways also provides a means to characterise the role and properties of this poorly understood protein modification.

POS-THU-062

FROM BINDING PROTEIN TO ENZYME: INVESTIGATING THE EMERGENCE OF CYCLOHEXADIENYL DEHYDRATASE ACTIVITY FROM NON-CATALYTIC ANCESTRAL PROTEINS

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Introducing catalytic function into non-catalytic protein scaffolds remains a major challenge in the field of enzyme design. Using ancestral protein reconstruction, we investigated how nature navigates this problem by characterising the evolutionary trajectory from a non-catalytic solute binding protein (SBP) to a catalytically active cyclohexadienyl dehydratase (CDT). Functional characterisation of extant homologs of CDT and reconstructed ancestral proteins reveal that CDT evolved from an arginine-binding protein via an intermediate of unknown function. The mutations required to introduce CDT activity into a non-catalytic ancestral protein were identified using site-directed mutagenesis and directed evolution. Crystal structures and molecular dynamics simulations highlight the structural basis for the emergence of catalysis in the SBP fold and indicate that a change in the conformational landscape of the proteins is likely to have contributed to the evolution of CDT activity.

POS-FRI-063

MODELLING THE EVOLUTION OF PROTEIN STRUCTURE

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While sequences in protein super-families can be remarkably diverse, even the most evolutionarily distant variants retain structural features. Analyses of remote homologues must deal with this sequence diversity. We hypothesize that ancestral reconstruction may benefit from the guidance of a *model* of structural evolutionary change, which explains the sway evolution has on structure. Dayhoff's point accepted mutation matrix expresses what amino acid substitutions are accepted by natural selection. We are now at a stage when a sufficient number of protein structures have been determined to develop *structural* 1-PAM matrices. Here, we use the PDB and two well-known structural descriptors, secondary structure and relative solvent accessibility, to develop such matrices. We compiled clusters of proteins with sequence identity higher than 85%, resulting in to 592 proteins, falling into 75 clusters. On the basis of the same 592/75 dataset, we calculated the 1-PAM matrices for amino acids, three- and seven-class secondary structure, and two-class relative solvent accessibility. Reassuringly, the correlation between the 592/75 amino acid 1-PAM matrix and the Dayhoff matrix is high at 0.89, which suggests that the structural 1-PAM matrices also capture evolutionary change on par with the interval of the amino acid 1-PAM. The following results stand out. First, substitutions between helix and beta-sheet are improbable according to 1-PAM, but probabilities range 0.002-0.017 for substitutions between bend or turn, helix, and beta-sheet. The seven-class secondary structure matrix reveals non-obvious tendencies between uncommon secondary structure classes; at a longer timeframe, 250-PAM reveals that helix and beta-sheet substitute at 15% probability. Second, exposed and buried status changes with 0.006 and 0.03 probability, respectively, in-line with the general observation that exposed*amino acids* mutate freely, but the exposed *status* itself is conserved. We are currently using these structural evolutionary models to improve sensitivity in the analysis of families that employ structurally common but sequence-diverse interaction interfaces, and in the reconstruction of ancestral proteins dating back a billion years.

POS-WED-064

ANALYSIS OF SILK GLAND TRANSCRIPTOME AND SILK MECHANICAL PROPERTIES OF THE AUSTRALIAN GOLDEN ORB WEAVERS NEPHILA PILIPES AND *NEPHLIA PLUMIPES*

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Spider silk is the world's toughest biological material yet very few spider species silks have been characterised. We analysed both the silk gland transcriptome and the silk mechanical properties of the exceptionally large Australian rainforest golden orb weaver *Nephila pilipes* in comparison to the related garden variety spider *Nephila plumipes*. Illumina next-generation sequencing of the major ampullate gland coupled with LC-MS/MS of the solid silk fibre identified highly expressed genes within the gland that may be involved in the formation of the silk fibres from the soluble to solid state, as well as proteins that may contribute to stabilization of the fibre. Biomechanical analysis of the two silk threads indicated *Nephila pilipes* had significantly tougher silk with higher strain capacity than its smaller congener, producing threads with toughness up to 305 MJ/m3. Within *N. pilipes*, smaller silk fibrils were produced by larger spiders, yielding tougher threads whose fibril diameter was negatively correlated with silk toughness. In contrast, while spider size was correlated with thread diameter in *N. plumipes*, there were no clear patterns relating to silk toughness, which suggests that the differences in properties between the silk of the two species arise through differing molecular structure.

POS-THU-065

A MOTOR MECHANISM FOR MTRD

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With over 106 million cases reported yearly, gonorrhoea is a major sexually transmitted infection for which no vaccine is available. Multidrug antimicrobial resistance in the gram-negative bacterium *Neisseria gonorrhoeae* is mediated in large part by the three-part MtrCDE efflux system. Molecular recognition and drug efflux in this system are powered by the RND membrane transporter MtrD. MtrD is a homologue of the well-characterised AcrB from *E. coli*. Crystallographic studies of AcrB suggest that drug binding and export is coupled to the import of protons across the inner membrane via a charge relay in the transmembrane region. In this way, the entropic cost of pumping drugs against the concentration gradient and out of the cell is paid by importing protons along the electrochemical gradient from the periplasm to the cytoplasm. By altering the charge state of the residues implicated in proton transfer, this coupling can be observed via molecular dynamics simulation of MtrD, allowing the structure-function mechanism of this motor protein to be elucidated. Here we link key proton-generated changes in the motor domain to transporter function, suggesting that a similar mechanism is in play in *N. gonorrhoeae* as hypothesized in the *E. coli* AcrB.

POS-FRI-066

CHARACTERISATION OF A METALLOENDOPEPTIDASE (PEPO) FROM *STREPTOCOCCUS PYOGENES*

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Streptococcus pyogenes, also known as Group A *Streptococcus* (GAS), are Gram-positive, β-haemolytic human pathogens that are able to cause infections ranging from the acute (e.g. bacterial pharyngitis, impetigo) to the severe (e.g. streptococcal toxic shock syndrome, necrotising fasciitis). Their significant impact on society makes it crucial to study the virulence factors that contribute to their virulence. Recently, PepO, a predicted zinc-binding metalloendopeptidase with sequence similarity to the M13 peptidase family, has been implicated as a virulence factor. Previous studies of PepO from other streptococcal species have found that it inhibits the classical complement pathway and binds to the C1q complement protein, plasminogen and fibronectin. In GAS, PepO is found to be a quorum quencher that mediates intercellular signalling through the degradation of active short peptide pheromones of the Rgg2/3 pathway and its expression is regulated by the CovRS system. In this study, I aimed to structurally characterise PepO both as an apoenzyme and a holoenzyme with the zinc (II) metal ion cofactor. PepO was expressed as fusion proteins with N-terminal affinity tags and purified via affinity chromatography and size exclusion chromatography. Experimental analyses suggested that PepO underwent proteolysis during the purification process and the addition of a metalloprotease inhibitor, 1,10-phenanthroline, prevented further degradation. Based on these results, I hypothesised that autocatalytic proteolysis may be a novel regulatory function of PepO. Future work include further optimisation of the purification conditions and crystallisation of PepO in both active and inactive forms for structural characterisation.

POS-WED-067

STRUCTURE-FUNCTION PROPERTIES OF NOVEL FIMH ADHESINS FROM MULTIDRUG RESISTANT *ESCHERICHIA COLI* ST131

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Escherichia coli Sequence Type 131 (ST131) has recently emerged globally as a major cause of multidrug resistant urinary tract and bloodstream infections in hospitals and the community. FimH is a well-studied *E. coli* adhesin with a critical role in extra-intestinal pathogenesis and is a target for new therapies for urinary tract infections (UTIs). Previous studies on non-ST131 uropathogens have revealed that single amino acid changes outside the mannose binding pocket of FimH can have a significant effect on adhesin function. Here we examine novel FimH variants found in the majority of clinical ST131 isolates that have not been previously studied. Using sequence-based analyses and molecular dynamics (MD) we are investigating the effect of FimH sequence diversity on adhesin structure. Preliminary MD findings and structural comparisons of the three most dominant ST131 FimH variants suggest that amino acid mutations unique to ST131 can significantly alter the structural conformation of FimH and potentially its function. The most prevalent FimH type among clinical ST131 strains (FimH30) appears to allow for less interaction between its two domains when bound to mannose, suggesting that it adopts a more elongated conformation which is associated with a higher affinity for its natural ligand and may increase adhesion to bladder and intestinal cells. Adhesion and invasion findings support this hypothesis. This project aims to elucidate the unique structure-function properties of *E. coli* ST131 FimH variants, which may provide an explanation for the widespread success of this clinically important pandemic lineage. Our findings are expected to inform preclinical studies on novel oral FimH inhibitors that have been shown to inhibit ST131 adhesion.

POS-THU-068

ELUCIDATING THE STRUCTURAL BASIS OF EFFECTOR INDUCED SUSCEPTIBILITY IN THE *PARASTAGONOSPORA NODORUM* - WHEAT INTERACTION

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Parastagonospora nodorum, the causal agent of Septoria Nodorum Blotch, is a major necrotrophic fungal pathogen of wheat worldwide. *P. nodorum* secretes small cysteine-rich proteinaceous effectors (ToxA, Tox1 and Tox3) that interact with corresponding dominant host sensitivity gene products, promoting cell death and rendering the plant susceptible to disease. Whilst it is now understood that these effectors are required for the development of disease, there is little understanding of their function. Using yeast two-hybrid and co-immunoprecipitation assays the effector protein Tox3 has been shown to interact with basic and acidic wheat pathogenesis-related 1 (PR1) proteins. To understand the structural basis of this interaction we have developed protein expression and purification systems for the production of Tox3 and wheat PR1 proteins. We present here the in-solution biophysical characterisation of Tox3 and wheat PR1 proteins using multi-angle light scattering coupled with size-exclusion chromatography. We further present the first crystal structure of an acidic wheat PR1 protein. This data provides structural and mechanistic insights into the interaction between Tox3 and wheat PR1 proteins.

POS-FRI-069

PROTEIN HANDLING AND ASSAY DEVELOPMENT THROUGH THE USE OF POLYMERIC METAL CHELATION COUPLING

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Immobilisation of proteins onto solid supports is a necessary element of many applications used in protein science. Traditionally, proteins are adsorbed onto surfaces by passive means or by a variety of covalent chemistries that can adversely impact both the structure and function of the immobilised protein. New generation applications, solid-phase surfaces and miniaturisation, together with increasingly complex assay platforms continue to challenge the conventional methods used for protein surface attachment. Anteo Technologies has developed an alternative approach that utilizes a metal nanopolymer, chelation-based surface chemistry. This approach relies on cationic metal polymers in aqueous solution that bind to synthetic surfaces with electron donating potential, resulting in the formation of nanometer thin coatings that are very strong and stable. The polymeric metal ions chelate and bind by multiple avidity points to both the surface and to biomolecules, thereby acting as a molecular binder. We demonstrate the technology's versatility and effectiveness to simply and gently bind proteins onto most of the solid support surfaces typically used in ELISAs, antibody coupled bead based assays, lateral flow assays, bioprocessing and biosensor applications for a broad spectrum of protein science applications.

POS-WED-070

RATIONAL DEVELOPMENT OF NOVEL ANALGESICS FOR THE TREATMENT OF CHRONIC PAIN: STRUCTURE-FUNCTION STUDIES OF AN ENGINEERED $Na_V1.7$ BLOCKER

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Neurotoxins are of interest as lead molecules for the development of pharmaceuticals. Huwentoxin-IV (HwTx-IV) is a spider-venom peptide. This neurotoxin is one of the most potent human-$Na_V1.7$ ($hNa_V1.7$) inhibitors described to date. $Na_V1.7$ is a subtype of voltage-gated sodium channel that is involved in the propagation of pain signals, and it is a validated analgesic target. HwTx-IV inhibits $hNa_V1.7$ with an IC_{50} of ~20 nM but inhibits the cardiac subtype $Na_V1.5$ with far lower potency. In this study, a rationally-designed, optimized, triple-mutant HwTx-IV analogue (oHwTx) was produced recombinantly via expression in the periplasm of *E. coli*. The mutant has significantly increased potency against $hNa_V1.7$ (IC_{50} = 0.4 ± 0.1 nM) without increased potency against $hNa_V1.5$. Neither its activity against other subtypes nor its structure has been characterised. Bacterial expression enabled production of uniformly $^{15}N/^{13}C$-labelled recombinant peptide for structure determination using multidimensional heteronuclear NMR spectroscopy. Additionally the activity of the recombinant peptide on various Na_V channel subtypes was measured via electrophysiology and FLIPR. In parallel the analgesic efficacy of the peptide was investigated using a rodent pain model. oHwTx and similar peptides from the same family inhibit Na_V channel activation by binding to the voltage sensor (VSD) of domain-II (DII). Here, we applied NMR-based methods to probe the atomic details of the interaction between gating-modifier venom peptides and isolated DII-VSD of several hNa_V subtypes. The knowledge gained from this work will lay the foundation for ligand-channel structural studies and provide a platform for rational engineering of subtype-selective inhibitors of $hNa_V1.7$, as novel analgesics.

POS-THU-071

CHARTING THE sORF-OME OF *ARABIDOPSIS THALIANA*

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Many small ORFs (<= 100AA) are often missed in genome annotations. To overcome this limitation Hanada *et al.* (2013) performed a large-scale study in *Arabidopsis thaliana* using *in silico* high coding potential as the criteria to identify putative intergenic coding regions. We have extended their study by employing ribosome profiling; the transcriptome-wide sequencing of ribosome protected mRNA fragments, which has greatly facilitated the detection of putative small coding ORFs. Using publicly available (Juntawong *et al*. 2014, Liu *et al.* 2013) and in-house generated Ribo-seq data, we charted the sORFome of *Arabidopsis*. We exploited the property of lactimidomycin to cause ribosome stalling at sites of translation initiation to identify cognate and alternative translation initiation sites with sub-codon to single-nucleotide resolution. Based on this ribosome protected fragment (RPF) signal, we were able to validate the recent discovery of miPEPs in primary miRNA transcripts of *Arabidopsis*(Lauressergues *et al*. 2015). Furthermore, we discovered many other putative coding sORFs in different categories of ncRNAs. The latest*Arabidopsis thaliana* annotation (Araport11, June 2016) also reports on 726 novel transcript regions (based on tissue-specific RNA-seq libraries from 113 datasets). In these novel transcribed regions, many (s)ORFs were also clearly delineated. We also corroborated these findings with matching mass spectrometry data. We first evaluated several extraction protocols on flower buds and other plant tissues for use in in LC-MS/MS. The methods described by Hernandez and Vierling (1993) and Conlon and Salter (2007) provided the highest protein concentrations. The Conlon and Salter method produced the greatest number of mass spectra for proteins <20 kDa. The Hernandez and Vierling method, despite producing a high protein yield, resulted in the lowest number of spectra. This proteogenomics approach greatly enhances the identification of *bona fide*translatable sORFs in plants. Hanada *et al.* (2013) Proc Natl Acad Sci U S A. 110:2395-2400. Juntawong *et al*. (2014) Proc Natl Acad Sci U S A. 111:E203–E212. Liu *et al*. (2013) Plant Cell. 25:3699-3710. Lauressergues *et al.* (2015) Nature. 520:90-93. Hernandez and Vierling (1993) Plant physiology 101:1209-1216. Conlon and Salter (2007) Methods Mol Biol. Humana Press, pp. 379-383.

POS-FRI-072

ARX HOMEOBOX INTERACTS WITH PICK1 AND IS PHOSPHORYLATED BY PROTEIN KINASE C ALPHA

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Aristaless-related homeobox (ARX) gene is a homeodomain transcription factor important for the development of forebrain, pancreas and testes. Mutations in *ARX* result in intellectual disability with or without comorbidities of epilepsy and brain malformations. We show that ARX is phosphorylated and using mass spectrometry and *in vitro* kinase assays we identify three sites of phosphorylation. Additionally, using yeast-2-hybrid we identify a novel ARX-interacting protein, PICK1 (Protein interacting with C kinase 1). PICK1 interacts with the C-terminal region of ARX, confirmed by CoIP. PICK1 is a scaffold protein known to facilitate phosphorylation of protein partners by PRKCA (protein kinase C alpha). We confirm that ARX is phosphorylated by PRKCA using *in vitro* kinase assays and a specific PRKCA inhibitor. To interrogate the effect of phosphorylation on ARX function (ie transcriptional repression) we generated and tested phosphoablative mutants by replacing serines with alanines. These changes did not impact cellular localization nor transcriptional repressor activity as measured by dual-reporter luciferase assays (HEK293T cells). Currently we are using RNASeq of HEK293T and pancreatic alpha cells overexpressing wild-type and mutant*ARX* to identify the functional effects of ARX phosphorylation. In conclusion, our data indicates that PICK1 interacts with the C-terminal region of ARX, and this transcription factor is phosphorylated via PRKCA. Mutations in this region of *ARX* cause a severe early onset seizure phenotype in affected children. It is attractive to speculate that in these cases the function of this transcription factor is disrupted due to compromised post-translational regulation of phosphorylation.

POS-THU-073

ANTIMICROBIAL, ANTICANCER AND INTERNALIZATION PROPERTIES OF CYCLIC GOMESIN: A MEMBRANE LINK

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The increasing number of resistant bacteria has been actively stimulating the search for alternative antibiotics. Antimicrobial peptides poses an interesting alternative as they are actively used by every species as a part of the innate immune system. Gomesin, a 18-residues peptide with two disulfide bonds arranged with ladder connection, is expressed in hemocytes of the Brazilian spider *Acanthoscurria gomesiana* and was shown to have potent antimicrobial activity towards Gram-negative bacteria and selective anticancer properties against melanoma cells. In a recent study a cyclic analogue of Gomesin was shown to be as active but more stable that its native form. In the current study we rationally designed a series of cyclic Gomesin analogues to improve its antimicrobial activity and investigate its mode-of-action. We successfully improved the activity by ~10-fold against tested Gram negative and Gram positive bacteria and melanoma cells without increasing toxicity. Mode-of-action studies revealed that cyclic Gomesin and its analogues act on bacteria and melanoma cells by targeting and disrupting cell membranes. Interestingly, cyclic Gomesin analogues were found to enter inside other tested human cell lines in a membrane-dependent but non disruptive way suggesting that membrane-disruption vs membrane-permeation is cell-type dependent and very likely determined by cell membrane composition. In summary, we have designed stable cyclic Gomesin analogues with potent antimicrobial activity and efficient cell-penetrating properties that can be used as stable scaffolds to deliver active sequences inside human cells. In addition, we have found that their mode-of-action and potency is cell membrane-dependent.

POS-THU-074

CHARACTERISATION OF THE BACTERIOPHAGE 186 IMMUNITY REPRESSOR BY MASS SPECTROMETRY, SAXS AND CRYSTALLOGRAPHY REVEALS A DODECAMER STRUCTURE WITH FLEXIBLY ATTACHED DNA BINDING DOMAINS

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The CI immunity repressor protein from bacteriophage 186 forms a wheel-like oligomer. To complement previous biophysical and *in vivo* reporter studies, the 186 CI protein was further studied using small angle X-ray scattering and electrospray ion mobility mass spectrometry. In addition, we have constructed a synthetic chimeric repressor protein, where the well characterised bacteriophage λ CI DNA binding domain is fused to the oligomerization domain of the 186 CI protein. The crystal structure of this chimeric repressor was solved in complex with DNA. Collectively, the results show that repressor dimers undergo further sequential oligomerization to form a dodecameric wheel. The DNA binding domains are shown to be connected to the oligomerization domains via a flexible linker which is thought to contribute to the protein's ability to bind to operators with different sequences and variable spacing. This complex becomes a scaffold that DNA can wrap around and loop on and off, to selectively control gene expression.

POS-FRI-075

ENGINEERED NANODISCS FOR STRUCTURAL STUDIES OF THE INTERACTION OF VENOM PEPTIDES WITH VOLTAGE-GATED ION CHANNELS IN A NEAR NATIVE ENVIRONMENT

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Voltage-gated ion channels are a superfamily of membrane proteins encoded by more than 143 genes in the human genome, making it one of the largest superfamilies of signal transduction proteins. These ion channels are recognised as among the most common and important, yet underutilised, drug targets. Unsurprisingly, there has been a surge of biophysical studies in the recent decades that aim to assess the interactions of these ion channels with potential inhibitors such as peptide toxins. Structural studies of membrane proteins are typically undertaken in detergent micelles. This model, however, presents uncertainties in its representation of the native phospholipid bilayer environment. Recently, soluble lipid bilayer platforms called nanodiscs (NDs) have been utilised to study membrane proteins in an attempt to achieve a more native environment. Nanodiscs consist of a membrane scaffold protein (MSP) wrapped around a lipid bilayer and embedded membrane protein, forming disc-like rafts that essentially mimic the flat phospholipid bilayer. NDs can stabilise membrane proteins in more native conformations, and can ultimately be used to derive more physiologically relevant functional and structural data. Here, we have used modified NDs appropriate for study by NMR spectroscopy to gain further insight into lipid binding and channel inhibition of the natural ligands of voltage-gated ion channels. The peptide-binding voltage-sensor domain (VSD) of a potassium channel, KvAP, was re-suspended in a nanodisc. We used NMR spectroscopy to study the interactions of a known peptide inhibitor, VSTx1, with the KvAP VSD. The study provides one of the first atomic resolution details of the binding of a peptide toxin to a VSD embedded in a lipid bilayer.

POS-WED-076

COLLABORATIVE CROSS CELL CULTURE (C4), AN IN VITRO MODEL FOR PHARMACOGENOMICS ANALYSIS

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One major aspect of personalised medicine is patient-to-patient variation in drug toxicity and response. Pharmacogenomics attempts to address this issue by identifying genetic factors that contribute to this variation. The Collaborative Cross (CC) mice consist of hundreds of octo-parental recombinant inbred lines, which account for greater than 90% of known variations in mice. This genetic reference population resembles the diversity seen in humans. Here we have established a cell culture collection of immortalised primary fibroblasts from the CC mice to overcome the problems of animal husbandry and to harness the high throughput power of cell culture models. Using the C4 collection, we have treated multiple cell lines with five different cytoskeleton-active drugs. Treated fibroblasts were stained for F-actin with phalloidin and DAPI and imaged using high throughput microscopy which were further analysed with a custom designed analysis pipeline. Different strains show varied phenotypic response to the five cytoskeleton-active drugs. These phenotype data can then be applied for quantitative trait locus mapping to find genes of interest that modulate the drug response.

POS-THU-077

PROFILING THE METHYLOME FOLLOWING DNA DAMAGE

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While DNA repair pathways and mechanisms are relatively well understood the role of the epigenetic landscape in this process has yet to be elucidated. It is conceivable that a more loosely packed chromatin environment is more susceptible to DNA damage while in contrast heterochromatin provides a protective environment. However the flip side of this is that euchromatin provides ease of access to DNA repair proteins while heterochromatin hinders access. In attempt to tease out the role of the epigenome in DNA repair we have profiled basal and post radiation global DNA methylation using the Illumina Infinium 450K arrays. This platform demonstrated that cells surviving this treatment have surprisingly stable methylomes with very few changes post exposure. Basal methylation profiles were characteristic of each cell line which may be indicative of their sensitivity to DNA damage or ability to repair DNA. This work provides the basis for further molecular characterisation of DNA repair within a chromatin context.

POS-FRI-078

GENOME ANALYSIS OF BARLEY LEAF RUST PATHOGEN, *PUCCINIA HORDEI*

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Puccinia hordei is an obligate biotrophic parasite that causes rust disease on barley. Up to 62% yield loss of barley caused by the pathogen has been recorded. To better study its pathogenesis, we sequenced its genome with the aim to search for effector and other pathogenicity related genes. 500bp and 3000bp DNA insert size libraries were constructed and sequenced on Illumina platform. The sequencing data was assembled with SOAPdenovo into 15,913 scaffolds (127Mbp) of high continuity (N50 22Kbp). The genome assembly is shown to be highly complete by CEGMA, a pipeline that measures presence of eukaryotic core genes. About 55% of the genome is reported as repeat, resembling the repeat contents of other rust pathogen species. We predicted gene models by using RNA-Seq of plant leaf tissues infected by the pathogen. 36% of the RNA-Seq reads could be mapped to the assembled genome and about 29k gene models were predicted based on the mapping. Functional annotation of the gene models shows hundreds of interesting candidates for effector proteins. The high quality genome assembly and gene annotation provides a good reference genome for comparative genomics and will facilitate identification of virulence related genes.

POS-WED-079

TRANSCRIPTOME PROFILING AS AN INITIAL STEP TOWARDS IDENTIFYING DEFENCE GENES TO *ASCOCHYTA LENTIS* IN LENTIL *(LENS CULINARIS)*

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Ascochyta blight, a major lentil disease caused by *Ascochyta lentis*, inflicts ~16.2 million dollars of economic loss annually on the Australian lentil industry. The most effective, economic and environmentally sustainable strategy is achieved by using A. blight-resistant lentil cultivars. Broadening the current knowledge of the lentil molecular defence response mechanisms would assist in identifying and improving resistant germplasm. Changes in the gene expression patterns in response to inoculation with *A. lentis* were examined in a resistant (ILL7537) and susceptible (ILL6002) genotypes via ion torrent RNA sequencing (transcriptome profiling). Samples were collected and compared at 2, 6 and 24 hours post inoculation (hpi) with either a highly aggressive isolate (ALP2) or H2O treatment. The transcriptome was assembled and annotated to predict open reading frames, known genes, protein families and molecular functions. The differentially expressed (DE) genes were categorised based on their annotation into three main physiological classes: primary defence response, induced defence response and necrotic structural defence response. The highest number of DE genes was found between the resistant and susceptible genotypes, in particular at 24 hpi, which correlates with the differential physiological response to the pathogen. This study describes a comprehensive transcriptome analysis of lentil defence response to *A. lentis*. Major defence related genes, functions and pathways were identified and their specific expression in the resistant genotype was revealed.

POS-THU-080

EPIGENETIC PROFILING TO STUDY THE RESPONSE TO SALINITY OF BARLEY *(HORDEUM VULGARE)*

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Soil salinity is a major cause of yield loss in barley and other crops across the world. Salt concentration in the soil changes with position, depth, seasonal progression and farm management, and can reduce yield even at relatively low concentrations. During growth, plants need to adapt to these variations in salt levels in a quick and dynamic manner. This requires physiological adjustments, including osmotic balance, tolerance of Na+ toxicity or Na+ exclusion through leaves. We aimed to examine whether physiological adaptation was driven by epigenetic changes in genomic DNA. Methylation-sensitive Genotyping By Sequencing was used to screen the genome of five barley varieties exposed to increasing salinity levels for salt-specific epiloci. We found a number of epimarkers that collectively allowed highly significant separation of controls from plants exposed to salt stress. These epimarkers may be involved in regulation of genes crucial to barley adaptation to salinity. The implications of these findings for the selection of salt tolerant lines in commercial breeding efforts are explored.

POS-FRI-081

ENRICHMENT OF EPITHELIAL EXOSOMES TO INCREASE THE SPECIFICITY OF CIRCULATING MICRORNAS AS BIOMARKERS FOR COLORECTAL CANCER

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Colorectal cancer (CRC) is a leading cause of cancer related death in Australians but, if detected early, is curable by resection. More sensitive and specific methods are being developed to enhance detection in screening programs. To identify biomarkers, changes in circulating microRNAs (miRNA) between control and CRC were identified. As CRC patients often present with co-morbidities including cardiac disease, hypertension and diabetes, it is notable that some CRC associated miRNA have also been proposed as biomarkers for these other diseases. Epithelial Cell Adhesion Molecule (EpCAM) is a cell surface molecule expressed on epithelia and epithelium-derived exosomes. EpCAM antibody labelled beads were used to capture epithelial tumour exosomes from serum to enrich for CRC associated miRNA miR-17, miR-19b, miR-20b, miR-21, miR-25, miR-186 and miR-486. Relative miRNA levels were determined by real time RT-PCR and were compared to unenriched sera in a cohort of 147 patients comprising 46 non-CRC controls (C), 51 advanced adenoma (AA), 32 stage III (SIII) and 18 stage IV (SIV) CRC patients. Significant differences in miRNA levels were seen in C participants between those with or without co-morbidities. The standout miRNA was miR-21. EpCAM enrichment enhanced specificity for CRC (ANOVA $p<0.05$) even when co-morbidities were taken into account. Poorer survival occurred in CRC patients with high EpCAM-enriched miR-21 levels (>0.004) encompassing all SIV and 78% SIII patients ($p<0.05$). Survival was poorest in patients with high miR-21 levels and low miR-186 levels, including 89% SIV patients ($p<0.001$). Results show promise that simple exosome enrichment may help to increase diagnostic specificity and that co-morbidities can influence miRNA levels in blood.

POS-WED-082

FURTHER ASSESSMENT OF EXOME WIDE UVR SIGNATURES IN MELANOMA

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C>T substitutions at dipyrimidine sites dominate the melanoma genome. We analyzed the exomes of spontaneous and neonatal UVR-induced murine melanomas, noting a dramatic change in the genomic footprint at C>T substitutions only after a single previous exposure. It suggested a preference for particular neighbouring pyrimidines at C>T SBSs, rather than “any” pyrimidine. In order to compare our findings with human melanoma genome and to confirm the kind of pyrimidine dimers most likely to drive melanoma, we re-analyzed published exome-wide footprints in human melanomas that were stratified in terms of likely levels of sun exposure. We utilized the Wellcome Trust Sanger Institute (WTSI) mutational signature package to detect mutational signatures in a particular cohort. Acral and mucosal melanomas were heterogeneous, but most C>Ts occurred in the context of 3’G, consistent with spontaneous deamination of the cytosine. C>Ts in the sun-exposed melanomas were statistically different from acral/mucosal lesions only for 5’T and 3’C. We hypothesize that C>Ts in melanoma either are overwhelmingly induced at TC or CC photoproducts, or that there are differences in DNA repair with respect to specific pyrimidines adjacent to the mutated cytosine that result in these signatures footprints at C>Ts in melanoma. We observed that the TpCpC signature appears to differentiate melanomas that have incurred some sun exposure, from those that have incurred none.

POS-THU-083

RNA INTERFERENCE: A PROMISING NATURAL MECHANISM FOR CROP PEST CONTROL

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Aphids are a major class of insect pests of economically important crops: the losses they cause are from direct feeding damage and transmission of many viral diseases. For example, the Green Peach Aphid (GPA, *Myzus persicae*) can transmit about 50% of plant viruses, and honeydew secretions enable growth of sooty moulds on host plants. These polyphagous pests are becoming increasingly resistant to some pesticides hence there is a need to develop other methods to control them such as RNA interference (RNAi). Its efficiency depends on appropriate selection of target genes. Since the nervous system is an effective target for chemical control, genes encoding neuronal signalling molecules were chosen for study. Using bioinformatics twenty-four genes were chosen as potential targets for aphid control, and the effects of their down-regulation were assessed after oral delivery of double stranded RNA (dsRNA) or host induced gene silencing. Silencing of some target genes via artificial feeding resulted in paralysis, or inability to moult, reducing their survival and/or reproduction. The results from in vitro RNAi studies allowed selection of promising candidate genes and initial in vivo experiment was conducted. The expected output of this research is a series of target genes that if down-regulated via artificial feeding or transgenic plants will confer GPA resistance to crop plants. The power of RNAi is its flexibility and the fact that it functions at the transcriptional rather than protein level. It can be applied to control other important aphid species, and also other plant pests.

POS-FRI-084

A FIRM GRIP: BIOMECHANICS OF THE IMMUNOLOGICAL SYNAPSE

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The cytotoxic T lymphocyte (CTL) is one of the key cell types responsible for eliminating cancer cells and as such, these cells have a great potential in developing anti-cancer immunotherapies. The cytoskeleton in the CTL comprises of critical machinery required for: shape change, cell division and migration. In particular, cytoskeletal remodelling in CTLs is vital for the formation of immunological synapses with tumour cells and subsequent delivery of cytolytic granules in order to induce cell death. Even though this is a physical interaction, the biochemistry behind the T cell receptor signalling pathway is well documented, little is known regarding the biomechanics that underlie CTL and cancer cell contact. Here we will use: a dual-micropipette aspiration assay and optical tweezers in combination with high resolution microscopy will be used to understand how mechanical forces generated by the CTL influences target cell interaction time and area, efficiency of granule release and ultimately its killing capacity.

POS-WED-085

THE CCC COMPLEX AND INTRACELLULAR ENDOSOMAL TRAFFICKING

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Copper metabolism MURR1 domain containing gene 1 (COMMD1) is the founding member of the COMMD protein family. This family consists of 10 proteins that are highly conserved and are found in all metazoan organisms. Each COMMD protein contains a C-terminal COMM domain, which facilitates protein-protein interactions, and an N-terminal domain which is thought to ascribe unique functions to each of the family members. COMMD1 is the most extensively investigated member of this family and has been shown to have roles in regulating NF-κB signalling and hypoxia adaptation. More recently, each of the COMMD proteins has been shown to interact with the coiled-coil domain containing proteins (CCDC) 22 and 93 to form the CCC-complex. This complex acts with other multisubunit protein complexes such as retromer and WASH to facilitate the recovery and recycling of transmembrane receptors from endosomal compartments to the plasma membrane. Given that each of the COMMD family members are able to interact with CCDC22 it is predicted that the CCC complex will have pleiotropic roles within the cell and its function will be required to maintain cellular homeostasis. To date this complex has been found to transport and interact with receptors critical to cellular development (NOTCH2), copper homeostasis (ATP7A) and cholesterol homeostasis (LDLR). In the current project we aim to characterise the interactions between members of the COMMD family, other CCC complex subunits and membrane lipids in order to further elucidate their role in membrane trafficking. Here we demonstrate that COMMD1 is able to interact directly with each of the COMMD proteins and also show that some COMMD proteins are able to interact with specific membrane phospholipids that likely control their intracellular localisation.

POS-THU-086

COMPARISON OF GENETIC BIOTINYLATION TAGS TO IDENTIFY INTERACTING PROTEINS OF GOLGI-LOCALISED SMALL G PROTEINS *IN VIVO*

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Recently, techniques have been developed to biotinylate interacting and proximal proteins within a living cell. These techniques include a promiscuous biotin ligase gene BirA* (BioID) and an engineered ascorbate peroxidase (APEX2) to provide genetic tags for the protein of interest. Due to the shorter half-lives of the intermediate reactive species, APEX2 is likely to have a smaller labelling radius than BioID. The application of both labelling techniques may provide information on the molecular neighbourhood of a given membrane protein. Our lab is interested in identifying the organisation of the molecular machinery that regulates membrane trafficking to and from the *trans*-Golgi network (TGN). Small G proteins of the Arl/Arf family are important regulators of membrane trafficking. Effectors of small G proteins interact only transiently with the active G protein and are therefore difficult to identify using standard technology. One Arl family member, Arl5b, regulates transport between the TGN and endosomes. To identify the effectors of Arl5b *in vivo* both BioID and APEX2 tagging systems were used. BirA*-Arl5b fusion protein and APEX2-Arl5b were expressed in HeLa cells and localisation was analysed by confocal imaging. Both fusion proteins were located to the TGN. Addition of biotin to BirA*-Arl5b transfected cells or APEX2-Arl5b transfected cells resulted in extensive biotinylation of proteins within the region of the TGN as detected by fluorescent Streptavidin staining. Immunoblotting detected a number of biotinylated proteins from transfected cell lysates. Purified biotinylated proteins from transfected HeLa cell lysates were analysed by mass spectrometry and the identified proteins examined by Ingenuity Pathway Analysis (IPA) to reveal potentially novel interacting partners of Arl5b.

POS-FRI-087

RECYCLING OF ALBUMIN IN KIDNEY CELLS AND MACROPHAGES

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Albumin and immunoglobulin G (IgG) are the two most abundant blood proteins, with long serum half-lives due to an efficient recycling system mediated by the neonatal Fc receptor, FcRn. FcRn-mediated protection of albumin and IgG has guided engineering of many novel therapeutics. FcRn is expressed in multiple tissues, but endothelial and hematopoietic cells appear to be the major sites of FcRn-mediated IgG recycling. Despite this, the role of FcRn in albumin homeostasis within these cells is not known. A better definition of FcRn and albumin trafficking within different cell types is required to optimise the serum lifespan of new recombinant protein therapeutics. Here we studied albumin trafficking in human embryonic kidney (HEK) derived cells that stably express FcRn. After FcRn-mediated internalisation, albumin traffics to early endosomes, and then recycling endosomes before disappearing from the cells. Moreover, albumin does not co-localise with markers of the late endosomes/lysosomes. Using an ELISA-based assay, we demonstrate that albumin is recycled into the culture medium in an FcRn-dependent manner. We also addressed the recycling of albumin in primary hematopoietic cells by using bone marrow derived macrophages (BMDM) isolated from mice expressing human FcRn, but lacking mouse FcRn. In BMDM, albumin is mainly internalised by macropinocytosis and traffics to structures positive for early endosomes markers but is not transported to recycling endosomes and lysosomes. Contrary to its FcRn-dependent recycling in HEK cells, internalised albumin is recycled independently of FcRn within BMDM. These findings highlight the importance of investigating a range of different cell types to understand the mechanisms of albumin recycling.

POS-WED-088

HUMAN WISKOTT-ALDRICH SYNDROME PROTEIN-INTERACTING PROTEIN (WIP) INTERACTS WITH YEAST HOF1P, A KEY REGULATOR OF THE YEAST CELL CYCLE

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Wiskott-Aldrich Syndrome Protein-Interacting Protein (WIP) interacts with Wiskott-Aldrich Syndrome Protein (WASP) to activate the Arp2/3 complex and promote the nucleation of branched actin filaments. Dysfunction of WIP and WASP in humans is implicated in a range of diseases such as the inherited immunodeficiency disease WAS (Wiskott-Aldrich Syndrome) and cancer. In *Saccharomyces cerevisiae* (baker's yeast), the Src Homology 3 (SH3) domain of Hof1p, a key regulator of the yeast cell cycle, binds proline-rich motifs in yeast WIP (verprolin/Vrp1p). In the absence of yeast WIP, the Hof1p SH3 domain has a toxic effect and appears to inhibit actin-dependent processes such as cytokinesis and endocytosis. Yeast cells lacking yeast WIP can be rescued by the expression of human WIP. If interactions between the SH3 domains of yeast Hof1p and its human equivalents and proline-rich motifs in human and yeast WIP have been conserved and mediate similar functions in actin-dependent processes, then the results of this study could aid in finding new treatments for WAS and elucidating the roles of WASP and WIP in tumor-suppression. The yeast two-hybrid system and *in vitro* pull-down assays using purified recombinant proteins were employed to test if the yeast Hof1p SH3 domain binds human WIP. We found that the yeast Hof1p SH3 domain interacts with two proline-rich motifs in the C-terminus of human WIP one of which is already reported to be important for human WIP function in yeast. This result indicates that these protein interactions have been conserved from yeasts to humans.

POS-THU-089

THE GTP-BINDING PROTEIN RAB31 MODULATES THE BIOLOGICAL PHENOTYPE OF BREAST CANCER CELLS BY AFFECTING TGF-β SIGNALLING

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In breast cancer, elevated levels of Rab31 in primary tumour tissue are associated with poor patient prognosis. Rab31 is a member of the large Rab protein family of the Ras superfamily of small GTPases. It regulates membrane traffic between the Golgi/trans-Golgi network and the plasma membrane and/or endosomes. In breast cancer cells, increased Rab31 expression enhances cell proliferation, while knock down of Rab31 mRNA levels by short hairpin RNA interference lowers cell proliferation rates. Additionally, increased expression of Rab31 leads to reduced adhesion of cells towards extracellular matrix proteins and decreased invasive capacity through Matrigel. As a control, a Rab31 mutant unable to insert into the Golgi membrane, due to deletion of the two C-terminal cysteine residues (Rab31-ΔCC), was overexpressed in breast cancer cells as well. In contrast to wild-type Rab31, overexpression of the functionally inactive mutant Rab31-ΔCC does not affect in vitro proliferation, adhesion, or invasion. Using microarray analyses and subsequent qPCR, Western blot and/or TGF-β activity assays for validation, Rab31 overexpression in breast cancer cells was demonstrated to modulate expression of other tumour biologically relevant genes, especially genes of the TGF-β superfamily including TGFB1, TGFB2, BMP7, SMAD6, FAS, and TNFSF10. Among others, Rab31 overexpression was found to strongly repress TGF-β, a known suppressor of breast cancer cell proliferation. Thus, Rab31 - depending on its expression level - may represent a major player in the change of the cell biological phenotype of breast cancer cells, i.e. a switch between a proliferative versus invasive phenotype, by mainly affecting TGF-β signalling.

POS-FRI-090

THE UNSTRUCTURED N-TERMINAL REGION OF DOUBLECORTIN X (DCX) MAKES CRITICAL CONTRIBUTIONS TO MICROTUBULE ORGANIZATION

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Doublecortin X (DCX) is a neuron-specific microtubule-associated protein that is essential for the cortical layering and neuronal migration in developing brain. Although DCX directly interacts with microtubules via its two structured doublecortin (DC) domains, the unstructured flanking N- and C-terminal regions of DCX have been proposed to play putative regulatory roles albeit by largely unknown mechanisms. We are investigating the contributions made by the unstructured DCX N-terminus (DCX 1-45) towards ability of DCX to organize microtubules and to regulate microtubule-related events. Whilst wild-type DCX expression leads to pronounced microtubule reorganisation into microtubule bundles in both neuronal and non-neuronal cell types, a progressive deletion analysis has revealed that loss of the first 29 amino acids was sufficient to prevent this impact on the microtubules. Furthermore, a DCX mutant lacking the entire N-terminus DCX-ΔN1-45 was unable to interact with microtubules as demonstrated in *in vivo* microtubule-binding assays. Expression of this DCX-ΔN1-45 mutant also impeded microtubule regrowth following nocodazole-induced microtubule depolymerisation. In parallel, we are also evaluating the contributions of phosphorylation of the N-terminus to regulation of DCX by the expression of phospho-mimetic or non-phosphorylated DCX mutants, again demonstrating an impact of these different DCX mutants on the DCX dynamics with microtubules as well as microtubule-bundling. Taken together, these results emphasize the critical regulation of DCX-microtubule interaction by DCX N-terminus and future work will tackle how these biochemical events contribute to regulation of neuronal migration.

POS-WED-091

STRUCTURE-FUNCTION ANALYSIS OF YEAST WISKOTT-ALDRICH SYNDROME PROTEIN

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Wiskott-Aldrich Syndrome Protein (WASP) and WASP-Interacting Protein (WIP) are human proteins that form a complex that directs the actin cytoskeletal rearrangements required for cell motility, cell-cell adhesion, endocytosis and cytokinesis. Budding yeast (*Saccharomyces cerevisiae*) has functional homologues of both WASP (Las17p) and WIP (Vrp1p). Yeast gene knock-out mutants deficient in Vrp1p (*vrp1Δ*) are viable at 24°C but not at 37°C (i.e. temperature-sensitive) and defective in endocytosis and cytokinesis. High copy number of the *LAS17* gene (resulting in Las17p over-expression) suppresses the growth defects of *vrp1* mutant cells. The aim of this study is to determine the mechanism of this high-copy-number suppression. Multiple fragments of Las17p were expressed under the control of the *LAS17* promoter carried on a yeast high-copy-number (2μ) vector in *vrp1Δ* gene knockout cells (devoid of Vrp1p) and tested for ability to suppress the growth defect. An N-terminal fragment of Las17p (aa 1-150) that binds Vrp1p, a large proline-rich central fragment (aa 151-530) that binds Src Homology 3 (SH3) domains and a C-terminal fragment (aa 531-633) that contains a WASP Homology 2 (WH2) domain that binds actin were individually insufficient for suppression, however a fragment containing both the central region and C-terminal region was sufficient for suppression. Within the central region aa 302-370 are required for suppression and within the C-terminal region the WH2 actin-binding domain and a second sequence of as yet unknown function are required for efficient suppression. Current efforts aim to elucidate the protein interactions responsible for suppression. The authors acknowledge the financial support of ARC Discovery Project grant (DP110100389).

POS-THU-092

QUORUM QUENCHING, IMUNOMODULATORY AND CHEMOPROTECTIVE MEVALONOLACTONE FROM *GUNDELIA TOURNEFORTII* L. SUPPRESS ENTEROPATHOGENIC *E. COLI* PATHOGENESIS IN HUMAN ENTEROCYTES

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Chemical investigation of the dichloromethane extract of *Gundelia tournefortii* L. afforded mevalonolactone by gas chromatography-tandem mass spectrometry. The mevalonolactone fraction was further studied for quorum quenching against enteropathogenic *Escherichia coli* (EPEC) infection and virulence on human enterocytes (CRL-1831) including cytoprotective and immunomodulatory activities. Significant downregulation of expressed EPEC virulence genes, *eaeAg* (intimin), *escC* (type III secretion biogenesis), and *tir* (translocated intimin receptor), was observed by qRT-PCR compared to controls ($P < 0.05$). The fraction did not exhibit cytoxicity in CRL-1831 (IC50 >100 μg/mL). EPEC infection challenge of CRL-1831 previously treated with the non-toxic fraction demonstrated inhibition of pathogen-induced apoptosis by downregulated expression of the early apoptosis marker *cjun*, accompanied by upregulated expression of the anti-apoptotic factor *A20*. Expression of *huGal1-R* in EPEC-infected set-ups was not affected suggesting non-disruption of facilitated bacterial clearance. Moreover, the fraction also showed anti-inflammatory activity by significantly downregulating the expression of pro-inflammatory *IL-8* and *TNF-α* cytokine genes compared to controls ($P < 0.05$). Simultaneously, fluorescence photomicroscopy revealed cytoprotective activity against EPEC pathogenesis by blockade of intimin-mediated host cell attachment. LigandFit molecular docking of mevalonolactone showed binding in the EPEC TIR intimin binding region inferring the reason behind the impedance of attachment on CRL-1831 cells. These findings support evidence on the anti-pathogenic, anti-inflammatory and chemoprotective activities of mevalonolactone from *G. tournefortii*L. during host-pathogen interaction. To the best of our knowledge, this is the first report on the isolation of mevalonolactone from*G. tournefortii* L.

POS-FRI-093

DETERMINING THE MOLECULAR INTERFACE BETWEEN NUTRITION, CELL GROWTH AND TUMOURIGENESIS

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Organ growth requires coordination of nutrient availability with the precise regulation of cell number and/or cell size. We have shown that the 'nutrient sensing kinase' (Salt Inducible Kinase 2, dSIK2) regulates cell growth/size in Drosophila and that it acts via the potent oncogene MYC. In Drosophila and mammals, SIK2 acts as a 'fuel gauge', monitoring cellular levels of ATP and glucose. When ATP or glucose levels fall (nutrient starvation), SIK2 promotes glucose synthesis to produce cellular energy. Thus, SIK2 activity is required to promote cell survival under conditions of metabolic stress. Intriguingly, the risk of specific cancers increases in patients with metabolic dysfunction including obesity and diabetes. However, the mechanisms that link diabetes, obesity and cancer remain poorly defined. Exciting studies, utilising a Drosophila cancer model, revealed that tumour cells were more aggressive in flies fed a high sugar diet. Strikingly, knockdown of dSIK2 in tumours of animals raised on a high sugar diet decreased tumour growth and prevented metastatic spread. We hypothesise, in a tumour setting, dSIK2 activity is required to overcome metabolic stress to promote cancer cell growth. Therefore, we aim to understand how the nutrient sensing kinase, dSIK2, regulates organ size in healthy tissues and how dSIK2 contributes to tumour pathogenesis.

POS-WED-094

STRUCTURAL BASIS FOR THE ROLE OF SORTING NEXINS IN HOST CELL INVASION BY CHLAMYDIA TRACHOMATIS

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Sorting nexins (SNX) are a large and diverse group of endosomal-associated proteins that are involved in a variety of functions, including endosomal sorting and cell signaling. The best-studied subfamily of SNX proteins is the SNX-BAR subfamily, which are comprised of an N-terminal phosphoinositide binding PX domain, and a C-terminal BAR domain that promotes membrane curvature leading to membrane tubulation. One of the major functions of SNX-BAR proteins is tubular-based endosomal sorting from endosome to the trans-Golgi-network (TGN), and this process is tightly coupled with a trimeric protein complex called 'retromer'. Recently, members of the SNX-BAR family were found to be hijacked by the bacterial pathogen Chlamydia trachomatis, and were recruited to the surrounding membrane of the large vacuole called the chlamydial inclusion. Chlamydia secretes a host of so called inclusion proteins or 'Incs' via a type III secretion system that decorate the surrounding inclusion membrane. One of these proteins, inclusion membrane protein E (IncE), sequesters the SNX-BAR proteins SNX5, SNX6 and SNX32 by directly binding their PX domains via a short peptide sequence. We have determined the structure of the SNX5-IncE host-pathogen protein complex at high resolution using X-ray crystallography, and find that an extended IncE sequence forms a long β-hairpin structure that binds with high affinity to a conserved surface of the SNX5 PX domain. This work provides the first structural insights into the function of the numerous chlamydial inclusion proteins required for host infection, and also the first example of how PX domains can regulate not only lipid interactions but also protein-protein association.

POS-THU-095

THE SIGNIFICANCE OF K-RAS MUTATION IN CANCER STEMNESS OF COLON CANCER

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K-ras is a well-studied oncogene and is commonly mutated in pancreas, lung and colorectal cancers. Cancers harboring K-ras mutations are difficult to treat due to drug resistance and metastasis properties. Cancer stem cells (CSCs) are considered a major cause of chemotherapeutic resistance and are thought to be responsible for tumor recurrence after therapy. K-ras mutations in CSCs are not well-understood. Here we demonstrate that K-ras mutations are related to CSCs' abilities to form tumor spheres and proliferate. We compared two colon cancer cell lines, HCT-116 and HT-29, the former is a K-ras mutated cell line and the latter is wild type. We found that HCT-116 cells treated with a K-rasG12C inhibitor S7333 formed significantly more tumor spheres than untreated cells (P=0.0474), while HT-29 (wild type) remained unchanged. However, the size of spheres (cell numbers per sphere) with S7333 treatment was smaller than the untreated cells, indicating their proliferation was inhibited. Consistent with this result, real time qRT-PCR showed that treated HCT-116 cells had lower Ki67 proliferation marker expression than untreated cells. Expression of Kras and self-renewal Lgr5 genes were significantly decreased, inversely TGF-beta1 was increased in the treated HCT-116 cells. Further, flow cytometry indicated that expression of CD133 increased and CD44 decreased in treated HCT-116 cell compared with HT-29 cells. These finding reveal that K-ras mutations are related to stem cell properties in colon cancer, and may therefore contribute to colorectal tumorigenesis and metastasis.

POS-FRI-096

MEASUREMENT OF CHROMOSOME LOSS RATES USING Y CHROMOSOME-LINKED FLUORESCENT REPORTER MOUSE

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The measurement of aneuploidy rates in mammalian cells can be slow and laborious. This problem has been alleviated in budding and fission yeasts by the development of colour-based mini-chromosome assays. This allows for millions of cells to be easily examined for mini-chromosome loss or non-disjunction by changes in colour sectoring in colonies on agar plates. We have applied a similar strategy by using gene-targeting to insert either green or red fluorescent reporter genes into the non-essential mouse Y chromosome. Transgenic mice have been generated with this reporter Y chromosome, which are healthy and fertile. In this study we show that we can detect fluorescent protein expression in multiple tissues in the adult male, as well as in the whole male embryo. To test the utility of the reporter mouse strains in measuring chromosome loss, we have crossed the mice to chromosome instability mutants. Male mid-gestation embryos from these instability mutant crosses show less fluorescent protein expression when compared to the reporter Y chromosome on the wild-type background. These mouse reporter strains may be useful in the screening of genetic and environmental factors that contribute to aneuploidy.

POS-WED-097

TARGETING CHROMOSOMAL INSTABILITY

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Chromosomal INstability (CIN), a hallmark of cancer, refers to cells with an increased rate of gain or loss of whole chromosomes or chromosome parts. As CIN is not found in normal cells, it offers a cancer-specific target for therapy. We generated a CIN model in Drosophila by knocking down the spindle checkpoint, and screened for candidate knockdowns that induce apoptosis only in CIN cells. Genes identified include those involved in the DNA damage/repair pathway, JNK signaling pathway, mitotic cytoskeletal regulation and metabolism. The screen demonstrates that it is feasible to selectively kill cells with CIN. CIN is linked to the progression of tumors with poor clinical outcomes such as drug resistance. CIN can give tumors the diversity to resist therapy, but it comes at the cost of significant stress to tumor cells. To tolerate this, cancer cells must modify their energy use to provide adaptation against genetic changes as well as to promote their survival and growth. We have demonstrated that CIN induction causes sensitivity to metabolic stress, with disrupted glycolytic flux and mitochondrial membrane potential. We showed that mild metabolic disruption that does not affect normal cells, can lead to high levels of oxidative stress and subsequent cell death in CIN cells because they are already managing elevated stress levels. We will present our latest work testing the effect of metabolic intervention in a tumour explant model. Altered metabolism is a differential characteristic of cancer cells, so our identification of key regulators that can exploit these changes to cause cell death may provide tumour-specific drug targets, especially for advanced cancers that exhibit CIN.

POS-THU-098

UVR AND ROS DAMAGE PROTECTION MEDIATED BY DCT EXPRESSION IN HUMAN MELANOCYTES

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The melanogenic enzyme Dopachrome Tautomerase (DCT) is involved in the formation of the photoprotective skin pigment eumelanin, and has also been shown to have a role in response to apoptotic stimuli and oxidative stress. To examine the effect of DCT on UVB DNA damage responses and survival pathways in melanocytic cells, knockdown experiments using melanoma cells, MC1R locus genotyped primary melanoblasts (MB) in monoculture and in co-culture with primary keratinocytes were carried out. Transduction with lentiviral vectors was used for DCT ablation and overexpression, and included MC1R WT and RHC homozygous strains known to be deficient in DCT. We found survival was reduced by DCT ablation and by UVB over time, whereas increased DCT protein levels enhanced cellular survival. DCT ablation reduced p53/pp53 proteins in most cases, while RHC MB cells displayed unchanged or decreased pp53. Overexpression of DCT in MB cells resulted in increased or unchanged p53/pp53 levels. Knockdown of DCT in melanoma cell lines and WT MC1R primary MB cells reduces the cell's ability to survive after UVB exposure, and alters DNA damage response protein expression, which reduces the cell's ability to repair UV-induced DNA damage. When compared to WT MC1R MB cells, RHC MC1R variant cells display vastly reduced expression of melanogenic proteins such as DCT, affecting sensitivity to UVB radiation and DNA repair pathways. When comparing co-cultures of WT MC1R-expressing MB cells with monocultures of MB cells only, a protective effect seems to be conferred by the keratinocytes to the MB, shown by increased cell survival when exposed to UVB.

POS-FRI-099

PHOSPHORYLATION OF THE BACE1 DISLL SORTING MOTIF IN HELA CELLS AND PRIMARY NEURONS MODULATES INTRACELLULAR TRAFFICKING AND REDUCES Aβ PRODUCTION

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BACE1 initiates the cleavage of the amyloid precursor protein (APP) to produce amyloid β-peptides (Aβ), a major component of amyloid plaques in Alzheimers disease. A key factor in the APP processing by BACE1 is the intracellular trafficking of both BACE1 and APP and the compartments where cleavage occurs. We have shown that BACE1 is rapidly internalized and traffics to the early endosomes and recycling endosomes in different cell lines (Chia et al., 2013). However, the mechanisms regulating endosomal sorting of BACE1 to the recycling endosome is less defined. A phosphorylated DISLL motif has been identified in the BACE1 cytoplasmic tail in vivo (Walter et al., 2001) and we explored whether this phosphorylation has a role in early endosome-to-recycling endosome trafficking. Expression of BACE1 phosphomimetic S498D and dephosphomimetic S498A mutants in Hela cells and primary neurons resulted in the S498A mutant predominantly localized to the early endosomes while the S498D showed increased levels in the recycling endosomes. To further investigate this difference in distribution, using antibody internalization assays, we showed that the S498D mutant traffics to the recycling endosomes from early endosomes at a faster rate compared with wild-type BACE1 while the S498A mutant traffics to recycling endosomes at a slower rate. Expression of BACE1S498A resulted in increased Aβ production while expression of BACE1S498D reduced Aβ production compared with wild-type BACE1. These findings indicate that phosphorylation of the DISLL motif can alter residency time of BACE1 in the early endosomes which in turns affects Aβ production. Currently, molecular machinery that regulate phosphorylated BACE1 to the recycling endosomes is being assessed.

POS-WED-100

TOWARD A MOLECULAR UNDERSTANDING OF THE INTERACTION BETWEEN MINT1 AND MUNC18A IN THE REGULATION OF SYNAPTIC VESICLE EXOCYTOSIS

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Munc-18-interacting (Mint) proteins are believed to act as adaptors and regulatory proteins involved in neuronal membrane trafficking. These are multi domain proteins composed of variable isoform specific disordered N-terminal regions, and a conserved C-terminal region that includes a PTB and two PDZ domains. Three mammalian Mint isoforms (Mint 1, 2 and 3) have been identified, but only the closely related Mint 1 and 2 are known to interact with the essential synaptic protein Munc18a and play redundant roles in the regulation of synaptic vesicle fusion during neurotransmission. Here, we identify the shortest region of Mint1 that is required for interacting with neuronal Munc18a, but not the Munc18c homologue found in other secretory cells. Munc18a controls the fusion of synaptic vesicles with the plasma membrane by binding the syntaxin-1 SNARE protein and regulating formation of the fusogenic SNARE complex. We find that the binding affinity of Munc18a and Syntaxin1 is significantly reduced in the presence of Mint1, suggesting an allosteric behaviour between Mint1 and Syntaxin1 upon binding to Munc18a. Moreover, our recent data show that the Mint1 peptide does not bind to Munc18a Δ317-333, where we have deleted a flexible loop within Munc18a proposed to regulate SNARE complex assembly. While speculative, our data suggests that Mint1 may function to control a key trigger point in SNARE complex assembly and vesicle fusion, and we are now pursuing further structural and functional studies to test this hypothesis.

POS-THU-101

TARGETING THE 'ACHILLES HEEL': INVESTIGATING THE MECHANISMS OF POLYMYXIN-INDUCED NEPHROTOXICITY

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With increasing incidence of antibiotic-resistant 'superbugs' globally and an estimated 10 million deaths per year by 2050, our world is dangerously close to reverting to the pre-antibiotic era. Polymyxins are 'last-resort' antibiotics against many Gram-negative 'superbugs'; however, nephrotoxicity is the major dose-limiting factor. Polymyxins are extensively reabsorbed by, and ultimately cause cell death in kidney tubular cells. However, molecular mechanisms underlying this nephrotoxicity remain poorly defined. Here we employed various molecular techniques to examine the mechanistic pathways which lead to polymyxin B (PMB)-induced cell death *in vitro* and *in vivo*. HK-2 (human proximal tubular) cells were treated with PMB (12.5-100 μM) for up to 24 h. Biomarkers for genome and chromosome instability were quantified using fluorescence microscopy. PMB-treated HK-2 cells showed significant increase in micronuclei frequency (up to ~18% total cell population when treated with 100 μM for 24 h, n=500, $p<0.05$), as well as abnormal mitotic events (over 40% in treated, n=30, $p<0.05$). Time-course studies were performed using a mouse nephrotoxicity model (cumulative 72 mg/kg over 48 h). Kidneys were collected over 48 hours and investigated for histopathological and DNA damage. Notable increases in γ-H2AX foci were observed in both HK-2 (up to ~44% cells with 5+ foci at 24 h, n=300, $p<0.05$) and kidney samples (up to ~25%, $p<0.05$). Consistent with these results, *in vitro* assays show PMB had high binding affinity to DNA. Altogether, our results indicate PMB has unwanted side-effects, causing DNA damage. This novel mechanistic information may lead to new strategies to overcome the nephrotoxicity of these important last-line antibiotics.

POS-FRI-102

GROWTH HORMONE INDUCTION OF THE IMMUNOTOLERANCE GENE H2-BL/HLA-G IS ESSENTIAL FOR SURVIVAL AFTER PARTIAL HEPATECTOMY

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Liver regeneration after partial hepatectomy is growth hormone (GH) dependent in rodents, and in C57BL/6 mice, we show that this is a consequence of induction of the potent immunosuppressive protein H2-Bl (homologue of HLA-G in human). We investigated partial hepatectomy in C57BL/6 mice harbouring different GH receptor (GHR) knockin mutants which delete key signalling domains. Striking mortality post-hepatectomy was evident only in mice lacking GH receptor (Ghr-/-), while GHR knockin mice disabled for all GH-dependent JAK2 signalling (Box1-/-), or for GH-dependent STAT5 signalling (Ghr391-/-), showed minimal mortality. Ghr-/- mortality was associated with decreased phospho-JUN expression, and increased apoptosis with elevated NK/NKT and macrophage cell markers. We had identified a set of GH-regulated genes triggered by a novel SRC pathway independent of JAK2, and within this set the Ghr-/- mice exhibited sharply decreased expression of H2-Bl, a key immunotolerance molecule that acts on the inhibitory receptors ILT2 and ILT4. We show H2-Bl is GH regulated in AML12 hepatocyte cells via SRC/JUN. We find that injury-associated innate immune attack by NK/NKT and macrophage cells is instrumental in failure of liver regeneration, and this can be overcome in Ghr-/- mice by adenoviral delivery of H2-Bl or by infusion of HLA-G protein. We identified that GH treatment of GH deficient patients' results in increased serum HLA-G. Our studies show that GH upregulation of H2-Bl reduces innate immune-mediated apoptosis after partial hepatectomy.

POS-WED-103

MUTANT IL7R ALPHA DIMERIZATION AND SIGNALING

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IL7Rα mutations described in T- cell acute lymphoblastic leukemia patients are characterized by insertion of unpaired cysteine in the extracellular juxtamembrane region. The mutant cysteine contributes to aberrant homodimerization of IL7Rα chains, Jak1/Stat5 constitutive signaling and induction of cell proliferation and survival, as previously reported. However, cysteine insertions in the wild type IL7Rα not always lead to signaling, even when dimerization occur. We now aim to better understand the mechanisms of dimerization and signaling of the mutant IL7Rα using site directed mutagenesis and IL7R functional assays. Considering cysteine alignment with IL7Rα intracellular regions involved in signal transduction, we constructed 13 different artificial mutants but only 3 triggered constitutive signaling. 241insCA showed the strongest signaling results and conferred proliferative advantage to BaF3 cells. In the other hand, 10 clinical mutants with cysteine in disparate positions were found functional in terms of constitutive signaling. Dimer projections of clinical mutant transmembrane sequences, using PREDDIMER, suggested alternatively the presence of a dimerization motif based on serine, SxxSxxS, aligned with the cysteine. However, substitutions of two or each of the three serines by alanine or isoleucine in the clinical mutant did not abrogate the constitutive signaling. Interestingly, prolines are the second most common amino acid inserted in IL7Rα clinical mutants. Proline substitution by alanine resulted in decreased constitutive JAK/STAT activation. Reintroduction of proline in different positions, either N- or C-terminal to cysteine restored the receptor signaling. In summary, our results suggest that besides cysteine alignment and possible presence of transmembrane dimerization motifs, other conditions have to be better investigated. Cysteine neighboring amino acids like prolines and their structural characteristics may be important to the dynamics of dimerization and signaling of mutant IL7Rα.

POS-THU-104

THE STRUCTURAL BASIS FOR GHR RECOGNITION BY JAK2

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Cytokine receptors play a pivotal role in many cellular processes. They initiate signalling cascades that regulate a vast range of vital physiological functions, including metabolism control, neural stem cell activation, inflammatory responses, bone development, as well as blood cell and immune cell development and growth. GHR is the member of the class I cytokine receptor family. This family does not possess an intrinsic kinase activity, however they bind JAK kinases via a conserved intracellular proline rich box1 motif, located a short distance from the cell membrane. A less conserved box2 sequence consisting of acidic and aromatic residues is located a short distance C-terminal of the box1. The box2 is also thought to play a role in interacting with JAKs and facilitating signal transduction from the receptor. The JAKs (□1150 amino acid) are multi-domain proteins possessing an N-terminal FERM (band 4.1, ezrin, radixin, moesin) domain followed by SH2, pseudokinase, and kinase domains. The FERM domain is required for receptor binding and the SH2 domain is also thought to be involved in receptor binding. Activation of the receptor results in JAK phosphorylation followed by further phosphorylation of multiple tyrosine residues on the intracellular domain of the receptor and subsequent phosphorylation of STATs. We sought to determine the structure of Box1-2 of GHR bound to FERM-SH2 domains of JAK2. We optimised a recombinant protein expression method resulting in high purity monodispersed solution with protein crystals identified from screening crystallisation conditions. Suitable crystals will be analysed to determine structural basis of JAK2 interaction with GHR.

POS-FRI-105

TIR DOMAIN POLYMERISATION OF ADAPTOR PROTEINS IN THE MYD88-INDEPENDENT PATHWAY

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Toll-like receptors (TLRs) are a family of membrane-bound pattern-recognition receptors (PRRs) that detect microbe-derived pathogen-associated molecular patterns (PAMPs) or host-derived damage-associated molecular patterns (DAMPs). Upon activation, TLRs undergo signall transduction that results in the release of transcription factors such as NF-κB, which induce expression of genes important for an innate immune response [1]. TLRs can signal through either the MyD88-dependant pathway, producing a higher-order protein complex known as the Myddosome; or alternatively through the MyD88-independent pathway, which is hypothesised to form a different higher-order complex. These adaptor proteins associate with each other via homotypic Toll-interleukin-1 receptor (TIR) interactions [2]. The TIR domains of the MyD88-dependent pathway MAL and MyD88 have already been characterised and we have observed that these proteins can form higher-order structures *in vitro* and *in vivo*(unpublished). To expand upon this work, we would like to look at higher-order assembly formation by the adaptor proteins TRAM and TRIF in the MyD88-independent pathway. TRAM has been purified and preliminary images of filaments have been taken using negative stain electron microscopy (EM). We would like to optimise TRAM polymerisation conditions and take images using cryo-EM for helical reconstruction. This would provide us a framework for understanding how TRAM undergoes polymerisation and possibly become an avenue for drug development. 1.Ve, T., J. Gay, N., Mansell, A., Kobe, B. & Kellie, S. Adaptors in Toll-Like Receptor Signaling and their Potential as Therapeutic Targets. Current Drug Targets 13, 1360-1374 (2012). 2.Ve, T., Williams, S. J. & Kobe, B. Structure and function of Toll/interleukin-1 receptor/resistance protein (TIR) domains. Apoptosis 20, 250-261 (2015).

POS-WED-106

PROTEOLYSIS OF EPHB4 IN PROSTATE CANCER PRODUCES A BIOACTIVE INTRACELLULAR DOMAIN FRAGMENT

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EphB4, a member of the largest family of receptor tyrosine kinases, is over-expressed in several epithelial cancers including 66% of prostate cancers (PCa), where it promotes tumour angiogenesis and increases cancer cell survival, invasion and migration. Our laboratory has identified 2 sequential protease-mediated cleavage events that liberate fragments of both the extracellular (ECD - 70 kDa) and intracellular (ICD - 50 kDa and 47 kDa) domains of EphB4 in 22Rv1- EphB4 over-expressing cells. The PCa-associated serine protease KLK4 was found to mediate the first cleavage event, releasing the ECD, with the remnant transmembrane fragment (50 kDa) being subsequently cleaved by γ-secretase to release the intracellular 47 kDa ICD fragment. Subcellular fractionation demonstrated that the 47 kDa fragment was present in the nuclear fraction suggesting nuclear translocation of this fragment. Both co-localisation and nuclear transport blockade by treatment with the α-importin inhibitor, ivermectin, demonstrated that nuclear translocation of the ICD was mediated by α-importin. Over-expression of the ICD fragment in PCa cells led to increased cell migration and proliferation as well as a changed cellular morphology. ICD over-expression also led to an increase in mRNA expression of *Lef1*, a known transcriptional regulator of the androgen receptor in the prostate. These data suggest that proteolytic production of the ICD leads to functional and potentially transcriptional effects in PCa cells and thereby provides the first evidence of novel mechanisms underlying the tumour-promoting effects of this important cancer-associated protein. The production of EphB4 fragments in PCa may be targetable by inhibition of proteolysis and this could be a potential novel avenue for anti-cancer therapies.

POS-THU-107

THE GTP-DEPENDENT STEP IS NOT INTEGRAL TO THE HETEROTRIMERIC EXTRA-LARGE Gα SUBUNIT, XLG2, IN REGULATION OF PLANT IMMUNE RESPONSE

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Heterotrimeric GTP-binding proteins (G proteins), consisting of Gα, Gβ and Gγ subunits, initiate cell-signaling upon ligand recognition by their receptors. They mediate multiple responses in eukaryotes. Animal Gα subunits function as molecular switches utilizing exchange of guanine nucleotides (GTP/GDP). In plants, Gα proteins evolved into extra-large Gα subunits (XLGs). XLGs retained interaction with the Gβγ dimer and form a heterotrimer at the plasma membrane. We found that Arabidopsis mutants lacking XLG2 and XLG3 were hyper-susceptible to diverse pathogens, indicating that XLG2 and XLG3 are required for plant disease resistance. Study of the quadruple mutants lacking G protein components indicated that XLGs and Gβγ interact functionally during immune signaling, confirming that XLGs are functional Gα subunits. However, from the biochemical studies and structural analysis, it remains questionable whether XLGs utilize GTP/GDP exchange to transduce the signal. We mutated the conserved GTP binding residue of XLG2 (XLG2T475N), rendering a protein incapable of GTP binding and found that immune response was not affected. We conclude that XLG2 functions independently of GTP/GDP exchange during immune response. We and others also established that XLG2 binds to several pathogenesis-related receptor-like kinases (RLKs), and mediates signaling in phosphorylation-dependent manner. We hypothesize that in plants XLGα subunits replaced the activation mechanism from GTP-dependent to phosphorylation-dependent.

POS-FRI-108

ANTI-MYELOMA ACTIVITY OF GOLD(I) COMPOUNDS

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Multiple myeloma (MM) is a clonal plasma B-cell neoplasm formed within the bone marrow. Despite being the second most common haematological malignancy after non-Hodgkin's Lymphoma, MM remains an incurable disease. Auranofin, a linear gold(I) compound known for its anti-arthritic properties has been recently trialled to treat a number of cancers including leukemia and lymphomas. Auranofin binds to the redox-sensitive selenocysteine residue present in thioredoxin reductase 1 (TrxR1) protein and therefore inhibits its activity. Previous studies in our lab demonstrated that auranofin exerts a significant anti-myeloma activity by inhibiting TrxR1 and increasing intracellular reactive oxygen species (ROS) levels. Although auranofin exerts a significant anti-cancer activity *in vitro*, its *in vivo* activity may be limited since it reacts readily and non-discriminately to protein thiols. In order to overcome these issues, a bis-chelated tetrahedral Au(I) phosphine complex called $[Au(d2pype)_2]Cl$ has been designed, which selectively targets selenol- and thiol- containing redox regulating proteins. In this study, we show that $[Au(d2pype)_2]Cl$ significantly inhibited TrxR1 activity in bortezomib-sensitive and resistant myeloma (RPMI8226 and U266) cells. $[Au(d2pype)_2]Cl$ treatment significantly inhibited cell proliferation and increased caspase-3 activity, an indicator of apoptosis, in bortezomib-sensitive and resistant myeloma cells. Currently we are investigating the underlying molecular mechanism for the anti-myeloma activity of this gold compound. Our findings indicate that this improved Au(I) compound exerts significant anti-myeloma activity in bortezomib-sensitive and resistant myeloma cells, suggesting it warrants further investigation *in vivo*.

POS-WED-109

COPPER INDUCTION OF LACCASES IN THE PLANT PATHOGENIC FUNGUS, *BOTRYTIS CINEREA*

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Botrytis cinerea, a fungal pathogen of grapevines, produces oxidative enzymes including laccases, during the infection of plant tissues. Laccases are reported to be induced by copper however it is not known if this is the case for *B. cinerea*. Three laccase genes are known (LAC1, LAC2 and LAC3) but gene specific inducers have not been fully investigated. This study investigates the expression of laccases in response to copper. Liquid cultures of *B. cinerea* were prepared in potato dextrose broth by inoculation of the media with 100 µLof spore suspension (10^5/ml). After 5 days mycelia were harvested and placed in a laccase inducing medium with a range of $CuSO_4$ concentrations (0 - 0.8 mM). Laccase activities in culture filtrates were determined after 2 days. Laccase activity was dependent upon copper concentration up to 0.6 mM and decreased at 0.8 mM indicating 0.6 mM is the optimum concentration of maximum laccase production. Mycelia were harvested for mRNA transcript analysis. LAC2 gene expression was similarly correlated with the copper concentrations in the culture medium with a 5-fold increase at 0.6 mM $CuSO_4$. There were no significant changes in LAC1 and LAC3 gene expression indicative that LAC2 is a copper-inducible laccase gene. Proteins from culture filtrates were concentrated using ultra centrifugal filters (30 KDa) and separated by SDS-PAGE. A single band corresponding to a 75 KDa protein was observed which may correspond to a copper inducible laccase. This is the first time laccase gene expression in response to copper has been investigated in *B. cinerea*.

POS-THU-110

CHARACTERISING DE NOVO PURINE BIOSYNTHESIS AS A PLATFORM TO ANTIFUNGAL DRUG DESIGN

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Cryptococcus neoformans is the leading cause of fungal meningoencephalitis and one of the major causes of death in immunocompromised individuals, particularly AIDS patients. Despite the existence of three commonly employed antifungals, mortality can reach almost 100% in some countries where the disease is highly prevalent, such as sub-Saharan Africa. It is therefore essential that we develop additional classes of antifungal drugs, particularly ones that are more effective than those currently available. Due to the shared eukaryotic physiology of fungi and humans, gross differences that can be exploited as drug targets are limited. An alternative approach is to exploit subtle differences in otherwise conserved pathways. Recent analysis by our group revealed *de novo* purine biosynthesis is essential for *C. neoformans* virulence. To facilitate our rational drug design program targeting *de novo* purine biosynthesis we have characterised and solved structures of several enzymes in the pathway to find potential other exploitable differences. One excellent example is adenylosuccinate lyase, an enzyme required for both the production of ATP and GTP via β-elimination of fumarate to convert SAICAR to AICAR and ADS to AMP. Given its dual activity and the short half-life of the substrate SAICAR it requires, it has been hypothesized this ADS lyase is involved in interactions with neighbouring enzyme in order to channel substrates. These interactions are thought to be transient, occurring in purine-depleted conditions, such as host infection. By determining if assemblies occur we hope to determine exposed domains that could be accessed by inhibitors.

POS-FRI-111

UNDERSTANDING THE MECHANISM OF (1,3; 1,4)-β-D-GLUCAN SYNTHESIS IN CEREALS

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Mixed linkage (1,3;1,4)-β-glucan (MLG) is a major non-cellulosic polysaccharide of the commelinid monocot cell walls and an important soluble dietary fibre component found in abundance in cereals. Yet despite its importance, relatively little is known about the molecular mechanism involved in the synthesis and assembly of this polysaccharide. Using functional genomics, the commelinid-specific Cellulose Synthase-Like *(CSL) F, CSLH* and *CSLJ* multi-gene families within the larger CAZy GT2 family have been identified as encoding the catalytic components of the MLG synthase enzyme (1-3). We have adopted a multi-disciplinary approach, including molecular, biochemical, proteomic and computational techniques to study CSLF6, the major MLG synthase in grasses. We have built homology models of various GT2 enzymes based upon the recently crystallised bacterial cellulose synthase BcsA (5-6) and have used molecular dynamics (MD) simulations to shed light on what protein features play a role in determining the specificity and sequence of the glycosidic linkages within this polysaccharide synthase family. We will present computational findings as well as experimental data relating protein structure to function. In addition, we will reveal biochemical evidence as to the modes of regulation of CSLF6. 1. Burton et al. (2006) Science 311, 1940-1942; 2. Doblin et al. (2009) PNAS USA 106, 5996-6001; 3. Little et al. (2016), submitted; 4. Smith & Stone (1973) Phytochem 12, 1361-1367; 5. Morgan et al. (2013) Nature 493, 181-186; 6. Morgan et al. (2014) Nature Struc & Mol Biol 21, 489-496.

POS-WED-112

IMPACT OF RESPIRATORY SYNCYTIAL VIRUS ON HOST CELL MITOCHONDRIA

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The World Health Organization estimates that Respiratory syncytial virus (RSV) causes 64 million infections and 160,000 deaths annually worldwide, making it the chief cause of viral pneumonia in infants, with more deaths each year than influenza. Although the clinical manifestations of RSV infection are known, the physiopathological mechanisms involved in disease are not understood. Recent proteomic studies in model systems indicate an impact of RSV on a number of nuclear-encoded mitochondrial proteins, implying a potential effect of RSV on mitochondrial function. Here we evaluate for the first time the effect of RSV infection on mitochondrial organization and function in A549 human alveolar basal epithelial cells. We additionally document RSV-induced changes in mitochondrial membrane potential, expression of oxidative phosphorylation complexes and metabolic functions over the first 24 hours of infection, and across different multiplicities of infection (MOIs). Mechanistic insights have been provided through use of different mitochondrial knock-out cell lines, probing the contributions of host cell mitochondrial health in RSV infectivity and viral production. Our results highlight the importance of host cell mitochondria in RSV pathogenesis thus paving the way for new therapeutic interventions and the development of anti-viral strategies.

POS-THU-113

LYSOPHOSPHOLIPID CONTENT OF AUSTRALIAN GROWN RICE

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Rice is traditionally soaked before being cooked in many parts of Asia because it is believed to improve the quality of cooked rice. Some studies have been done on the effect of soaking during parboiling or at elevated temperatures but soaking of unhulled rice during parboiling is quite different from soaking hulled and milled rice at room temperature. In this study, eleven Australian grown rice cultivars were soaked for 24 hrs at room temperature to ascertain the effect of soaking on extractable starch lipid, mainly on lysophospholipid (LPL), content. Lipids were extracted using 75% n-propanol and then LPLs were analysed using LC-MS. There was wide variation in LPL content between cultivars, particularly four red/black cultivars. Although soaking had a statistically significant effect on two of ten LPLs in rice cultivars, the effect was minor compared to cultivar differences.

POS-FRI-114

NON-COMBINATORIAL LIBRARY SCREENING REVEALS SUBSITE COOPERATIVITY AND IDENTIFIES NEW PEPTIDE SUBSTRATES FOR HUMAN NEUTROPHIL ELASTASE

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Human neutrophil elastase (HNE) has a well described bactericidal role and is also a key driver of the inflammation and parenchymal tissue damage that ultimately causes Chronic Obstructive Pulmonary Disease. To understand the enzyme's substrate selectivity and identify potential motifs for inhibitor design we synthesised a non-combinatorial (sparse matrix) library of tetra peptide substrates to screen against HNE. Sparse matrix libraries differ from the more common positional scanning approach in that they are able to discern positional enhancement or hindrance from the interaction of proximal amino acid sidechains in a given substrate. In particular we found a marked preference for Methionine sulfoxide as the second amino acid of the tetra peptide. This selectivity together with the profile's evident strong subsite cooperativity led us to carry out molecular dynamic simulation of preferred substrates in complex with HNE and identify the key elements of the HNE/substrate interface driving selectivity. Additionally, comparison of the most favoured substrates with known bacterial protein substrates of HNE revealed potential cleavage points and suggests a connection between the hypohalous acid and protease activities that macrophages use to destroy invading bacteria.

POS-WED-115

UNRAVELLING THE CAUSES FOR REDUCED GRAIN PROTEIN UNDER ELEVATED CO_2

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Atmospheric CO_2 [CO_2] is predicted to reach a concentration of ~550 ppm by 2050. This ~35% rise from a current [CO_2] of ~400 ppm is affecting plant performance. In wheat, e.g., above- and below ground biomass as well as grain yield is stimulated while grain N and thus protein concentrations are reduced, negatively affecting the nutritional and market value of grains. In order to unravel and overcome N reductions under elevated CO_2 (e[CO_2]), the following questions were investigated: (1) Are differences in root growth associated with differences in N status under e[CO_2]? (2) Does nitrate accumulate in leaves of e[CO_2]-grown wheat indicating impaired nitrate reduction? (3) Is N remobilisation from leaves into grains and expression of related genes affected by e[CO_2], thereby contributing to grain N reductions? Questions were tested on field-grown wheat under ambient (a[CO_2]) or e[CO_2] (~550 ppm) within the Australian Grains Free Air CO_2 Enrichment (AGFACE) program. Root growth was stimulated by e[CO_2], but was not related with grain N status. In contrast, the ratio of nitrate to total N in leaves was greater under e[CO_2] and was significantly related to decreased grain N. Also, a range of genes relating to N and C metabolism was up-regulated in senescing leaves under a[CO_2] but no such up-regulation was observed under e[CO_2]. We suggest that (1) CO_2 enrichment impaired nitrate reduction so that, despite sufficient N uptake, unassimilated nitrate accumulated in leaves and (2) CO_2 enrichment caused a greater demand for N translocation into the developing grain which was not met.

POS-THU-116

ENGINEERING THERMOSTABLE AND INDUSTRIALLY USEFUL CYTOCHROME P450 ENZYMES

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Cytochromes P450 show potential for use as biocatalysts in industrial processes because of their ability to catalyse a wide range of stereo- and regio-specific reactions that are difficult to achieve synthetically. However, there are currently many limitations to using these proteins including the relative lack of thermostability of the native forms. Previous studies have revealed that reconstructed ancestral P450s were significantly more thermostable than their extant descendants. To further investigate this phenomenon, twelve ancestor P450 proteins of increasing evolutionary age were inferred from extant enzymes of the CYP2 family, and characterised for their thermostability. The results show an overall trend of increased thermostability in proteins of an older evolutionary age. It is hypothesised that this stability is the result of an increased level of hydrophobic packing in the protein core. The next phase of this investigation is to assess the substrate range of these ancestral P450s to determine their catalytic capabilities and the trade-off between thermostability and promiscuity.

POS-FRI-117

PIGMENTS IN RUST FUNGI: BIOSYNTHESIS, ROLE AND EVOLUTION

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The rust diseases are caused by fungi and are among the most damaging of all plant diseases. The most characteristic feature of all rust fungi is the pigments that are produced in one or more spore forms, which give them a rusty appearance. The pigments are thought to protect rust fungi against UV radiation and oxidative stress, and possibly act as a virulence factor. Phytoene desaturase (585 aa, encoded by the *CrtI* gene) and lycopene cyclase (707 aa, *CrtY*) were identified as candidate genes for cytoplasmic carotenoid pigment biosynthesis in rust fungi. In the postulated pathway, phytoene desaturase conducts the desaturation of phytoene (colourless) leading to lycopene (red-coloured), and then lycopene cyclase cyclizes lycopene to generate *γ*-carotene or*β*-carotene (yellow-coloured). To obtain a better understanding of the pigment biosynthetic pathway in rust fungi, cytoplasmic pigment profiles of 32 wild-type and colour mutants of *Puccinia graminis* f. *sp. tritici* (*Pgt*), *P. g.* f. *sp. avenae* (*Pga*), *P. g.* f. *sp. secalis*, *P. triticina*, *P. coronate* f. *sp. avenae*, *P. hordei*, *P. striiformis* and *P. psidii* were determined by high performance liquid chromatography. Four carotenoids, including phytoene, lycopene, *γ*-carotene and *β*-carotene, were identified in the urediniospores of wild-type spores. Compared with the wild-type parents, yellow mutants in *P. graminis*, *P. triticina* and *P. hordei* contained less lycopene, but more *γ*-carotene and *β*-carotene. An albino mutant of *P. striiformis* accumulated only phytoene, indicating that the pathway was blocked between phytoene and lycopene. The candidate genes were amplified and sequenced. Variations of the sequences between *Pgt* and *Pga* were 1.54% (*CrtI*) and 3.25% (*CrtY*), respectively. Function of candidate genes will be verified by complementation studies.

POS-WED-118

EXPLORING THE MOLECULAR GENETICS AND CHEMISTRY OF TROPANE ALKALOID PRODUCTION IN SPECIES BELONGING TO THE AUSTRALIAN TRIBE *ANTHOCERCIDAEA*, FAMILY *SOLANACEAE*

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The mainly Australian tribe *Anthocercideae*, family *Solanaceae*, contains a number of genera which produce the valuable and medically important tropane alkaloids hyoscyamine and scopolamine, derived from the primary metabolite putrescine, the precursor of polyamines which are essential for plant growth and vitality[1]. Interspecific hybrids in the genus *Duboisia* are farmed commercially as a source of these medicinal alkaloids in Australia and overseas. *Duboisia* and other *Anthocercideae* genera such as *Cyphanthera* and *Anthocercis* are known also to produce other metabolites derived from putrescine, including toxic undesirable pyridine alkaloids and also calystegines. In the current project we are exploring links between the molecular genetics and production of these metabolites in *Duboisia* and *Cyphanthera* species, and hybrids thereof with the aim of identifying control points for targeted precision breeding to ensure maximal rates of diversion of primary metabolites into valuable tropane alkaloid metabolism without concomitant production of non-valuable metabolites or negative effects upon plant health. 1.Ryan SM, DeBoer KD, Hamill JD (2015) Functional Plant Biology, 42, 792_801.

POS-THU-119

IMPROVING PATIENT RESPONSE TO CANCER DRUG (CETUXIMAB) BY STOPPING TRAFFICKING OF RECEPTORS

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The efficacy of cetuximab in treating cancers is 10-20% due to de novo and acquired resistance. One of the notable mechanisms of action of cetuximab is inhibition of signaling, which is mainly tumourstatic. The tumourlytic mechanistic pathway is the induction of antibody dependent cell mediated cytotoxicity (ADCC). We hypothesize that if antigenic epitopes are arrested on the tumour cell surface by inhibiting their internalisation, increased tumour killing can occur. Our laboratory *in vitro* experiments showed significant SCC cell killing with cetuximab using dynamin inhibitors to stop ligand-receptor complex endocytosis. This increased killing, reversed innate resistance in resistant cells and increased killing of sensitive cells. We therefore tested our proposition *in vivo* using NSG mice. Since the NSG mice lack NK cells and other lymphocytes, we humanised their immune system after SCC tumour transplant. Thereafter, we introduced cetuximab/dynamin inhibitor combination therapy. The outcome showed greater statistically significant tumour cell killing in the treated than the control mice in both sensitive and resistant SCC tumours. We subsequently demonstrated that the target receptors of mAb therapy can be redistributed to the tumour surface and reverse tumour heterogeneity for therapeutic target in a Phase I proof-of-mechanism clinical trial. We are now progressing to Phase II combination therapy trials. The great prospect in this mechanistic highway is that it overrides most activating and other mutations that cause *de novo* or acquired resistance.

POS-FRI-120

CHARACTERISATION OF ROOT ARCHITECTURAL RESPONSES OF MUNGBEAN TO WATER DEFICIT

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Research into making agricultural systems more water efficient is gaining attention in recent years as the majority of climate change scenarios paint a deteriorating picture of fresh water availability. Agriculture accounts for in-excess of 70% of the worlds' freshwater usage and this figure is set to increase by another ~19% by 2050. Lack of freshwater availability has been described as the single biggest problem in meeting the ever-increasing global food requirement. Other abiotic stresses leading to crop losses include salinity, temperature, and chemical toxicity. However, of these, drought and salinity (often occurring in conjunction) are the most costly. Vigna radiata (mungbean) is one of the most important pulse crops in the world. They are one of the most economical sources of protein (24%); contain high levels of dietary fibre, essential amino acids including methionine and lysine, vitamins, minerals and only a small amount of oil. Mungbean has been commercially grown in Australia since the late 1960s and 1970s and it is believed annual production could see drastic increases if we are able to improve abiotic stress tolerance – particularly drought. The present study investigates physiological and morphological responses of differentially drought tolerant varieties of mungbean under regulated deficit irrigation (RDI) at the Queensland Crop Development Facility. Seed pre-treatment with a novel chemical referred to as ATW1124 was investigated as a putative enhancer of root systems architecture for the improvement of adaptability to water limiting conditions. Another pillar of the study was in simulating the effects of ATW1124 on mungbean in APSIM to determine potential impacts on production. Finally, RNA-Seq transcriptome analysis will reveal molecular mechanisms underpinning these responses.

POS-WED-121

SWEETENING THE DEAL FOR NARROW-LEAFED LUPIN (*LUPINUS ANGUSTIFOLIUS* L.): UNDERSTANDING THE QUINOLIZIDINE ALKALOID BIOSYNTHETIC PATHWAY

Frick K.M.[1, 2, 3], Foley R.C.[1], Kamphuis L.G.[1, 3], Siddique K.H.M.[3], Nambiar S.[4, 5], Trengove R.D.[4, 5] and Singh K.B.[1, 3]
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Narrow-leafed lupin (NLL) is a key grain legume crop in Australia that has recently gained recognition as a human health food. The grain is high in protein and dietary fibre whilst being low in fat and starch, and demonstrates a range of nutraceutical benefits. The value of the grain is however limited by the accumulation of quinolizidine alkaloids (QAs) - secondary metabolites which are unpalatable and sometimes toxic. Grain QA levels must remain below industry thresholds (<0.02%) in order to be used for food and feed purposes, however levels can vary considerably, sometimes exceeding these thresholds. Mechanisms of both QA production and environmental influence on this are poorly understood. This project makes use of newly available NLL genomic and transcriptomic data, in conjunction with molecular techniques, to identify and characterise genes involved in the biosynthesis of QAs. We have also developed a highly sensitive GC-MS/MS method for detecting NLL QAs, used to measure responses of QA production to environmental conditions in order to better understand how industry thresholds are exceeded. The findings from this project will serve to assist breeding programs and farmers to produce NLL grain that consistently meets QA thresholds, enhancing the value of a key Australian grain crop.

POS-THU-122

IRON BIOFORTIFICATION OF CHICKPEA

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Iron deficiency is one of the major micronutrient deficiencies worldwide and affects both developed and developing nations. Existing methods of alleviating this issue, such as supplementation and food fortification, are limited by the economic status of the targeted demographics. Biofortification presents a sustainable means to overcome this drawback. Iron biofortification via genetic modification has been done in several important crop species such as rice and wheat; however no work has been done on pulses despite their status as an important secondary staple. Pulses are rich in protein and micronutrients including iron, most of which is not bioavailable. This project focuses on the iron biofortification of chickpea via genetic modification. Chickpea is the world's second most important pulse crop and is consumed widely, particularly in India where anaemia is prevalent. In this project, a combination of nicotianamine synthase (NAS) and ferritin, which have been successfully used in rice and wheat biofortification, were used to transform chickpea half-embryonic axes through Agrobacterium-mediated transformation. Assessment of iron content in the T1 and T2 progeny was done using LA-ICP-MS (Laser Ablation-Inductively Coupled Plasma Mass Spectroscopy) and ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy). Preliminary results with the leaves and seeds show an enhanced iron accumulation of 3-fold in transgenic leaves and 1.3-fold in transgenic cotyledons compared to the non-transgenic controls.

POS-FRI-123

CAN-SEQ: A PCR AND DEEP SEQUENCING STRATEGY FOR IDENTIFYING NEW ALLELES OF CANDIDATE GENES REQUIRED FOR SYSTEMIC RNA SILENCING IN ARABIDOPSIS

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Forward genetic screens are used to identify the genes required for biological traits. However, new alleles arising in known genes can obscure the full repertoire of genes that are required for a trait. We carried out a forward genetic screen and identified 41 independent Root-to-shoot Transmission of Post-transcriptional gene silencing (rtp) mutants of Arabidopsis. To filter out rtp mutants that carried mutations in known or related genes, we developed a PCR-based sequencing approach called Can-Seq (Candidate gene-Sequencing). Leaves of up to 25 independent rtp mutants were bulked, and extracted DNA was used as template to amplify 47 candidate genes. PCR amplicons were bulked, sequenced and candidate mutations identified. PCR zygosity tests were used to identify the rtp mutant carrying each candidate mutation. 30 of the 41 rtp mutants carried homozygous mutations in one or more candidate genes. Complementation tests confirmed that the candidate mutations were the causative mutation in several rtp mutants. Importantly, 11 rtp mutants did not carry mutations in candidate genes, and therefore represent novel genes required for systemic RNA silencing.

POS-WED-124

FINDING THE SWEET SPOT: GENETIC AND CHEMICAL GENETIC SCREENS FOR SUGAR SIGNALLING IN PLANTS

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Photosynthesis provides the stored energy and the molecular building blocks for life on Earth. Sugars also have hormone-like properties in plants that regulate growth, developmental processes such as flowering time, affect pathogen sensitivity, and contribute to cell cycle progression. Coordinating photosynthesis in a multicellular, sessile organism requires integration of external cues with metabolism and transport processes across diverse cellular and subcellular compartments. This depends on multiple carbohydrate sensing and signalling pathways. We aim to understand how rhythmic sugar signals are sensed and decoded by plant cells. In particular, we are interested in how sugar and light signals interact in the context of photoautotrophy. Our previous research has shown that sugars produced from photosynthesis contribute to entrainment of the Arabidopsis circadian clock (Haydon et al., Nature 2013). The precise signalling pathways by which this occurs are not yet known, largely due to the genetic recalcitrance of sugar signalling pathways in plants. We are using novel tools and assays to better understand these and other signalling pathways and the downstream transcriptional networks.

POS-THU-125

PLEIOTROPIC REGULATORY LOCUS 1 IS REQUIRED FOR ROOT TO SHOOT TRANSMISSION OF RNA SILENCING IN *ARABIDOPSIS* EMBRYOS AND SEEDLINGS

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Post-transcriptional gene silencing (PTGS) of transgenes is graft-transmissible and acts systemically throughout plants. A forward genetic screen identified one mutant, *rtp1-1*, that showed delayed onset of systemic PTGS and a variety of developmental abnormalities. We used map-based cloning to identify *rtp1-1*, and found it to be a new allele of the previously described *PLEIOTROPIC REGULATORY LOCUS 1* (*PRL1*). Recently, it was reported that PRL1 is an RNA binding protein required for the accumulation of endogenous small regulatory RNAs in *Arabidopsis* shoot tissues or whole plants. *PRL1* is a member of a two-gene family in *Arabidopsis*, and we also characterized the *prl2-1* T-DNA insertion mutant and the *prl1*(*rtp1-1*) *prl2-1* double mutant for defects in systemic RNA silencing. PRL1 is also a core component of a plant defense pathway required for plant innate immunity, and we have characterized *prl1*, *prl2* and mutants defective in small regulatory RNA (sRNA) biogenesis for an altered response to the root-rot pathogen *Fusarium oxysporum* (*Fo*).

POS-FRI-126

LIVE AND LET DRY: *TRIPOGON LOLIIFORMIS* REGULATES AUTOPHAGY TO SUPPRESS CELL DEATH AND TOLERATE DESICCATION

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Over the next fifty years there will be a massive challenge to sustain an ever-increasing global population which is predicted to peak at 9 billion people in 2050. Further compounding this burgeoning population, climate-change forecasts predict increasing erratic weather globally and reduced crop yields in future environments. How do we fulfil this ensuing supply-demand gap? A small group of angiosperms termed resurrection plants have the ability to tolerate desiccation and return from a dormant state upon watering and may serve as a unique genetic resource for the generation of resilient crops that can survive and yield during unfavourable conditions. Here, we use, *Tripogon loliiformis*, a native Australian resurrection plant to answer the intriguing question of how resurrection plants maintain vitality in desiccated tissues? We show that *T. loliiformis*utilises a unique regulatory role for the non-reducing sugar trehalose in the activation of autophagy pathways during drying. Transcriptome, Gas Chromatography Mass Spectrometry and confocal microscopy analyses correlated trehalose accumulation with the onset of autophagy in dehydrating *T. loliiformis* shoot tissues. These results were supported *in vitro* with the observation of autophagy in hydrated *T. loliiformis* leaves following treatment with a 25 mM trehalose solution. Autophagy can promote survival of cells by removal of damaged organelles and misfolded proteins to suppress PCD and nutrient recycling to delay the onset of senescence. These findings illustrate how resurrection plants manipulate sugar metabolism to survive desiccation and may provide potential targets for the development of stress tolerant crops.

POS-WED-127

GENETIC ENHANCEMENT OF CHICKPEA VARIETIES FOR IMPROVED DROUGHT RESISTANCE AND PREDICTION OF YIELD PERFORMANCE USING APSIM MODEL UNDER SOIL BED GLASSHOUSE CONDITION

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Chickpea is an important nutritious pulse crop of high demand globally. Due to lower than expected yields in the Indian subcontinent as a result of climatic changes, Australia has an unprecedented opportunity to significantly increase chickpea production and export. While we have some natural advantages, increasing chickpea production also has some inherent challenges. Increasing climate variability and change including excessive heat and water deficit as well as the increasing incidence of pests/diseases are major risk factors that affect the industry. Forecasters predict that by 2070 there will be 40 % more months of drought in eastern Australia and conditions will be worse in a high-emissions scenario. Upon abiotic and biotic stress, plant cells accumulate high levels of misfolded proteins that can lead to cell death. To mitigate stress levels plants have evolved cytoprotective genes that help maintain proper folding of proteins and reduce stress-induced death. The BAG genes are a family of multifunctional stress protective co-chaperones that facilitate protein folding and are conserved in mammals as well as plants. Here we describe the development and assessment of elite GM chickpea varieties expressing BAG genes isolated from Arabidopsis thaliana and the Australian resurrection plant Tripogon loliiformis. An efficient regeneration and transformation system was established using Agrobacterium—mediated transformation of half embryonic axis of chickpea (variety Hattrick). Using this system we achieved transformation efficiencies of up to 3 %. In glasshouse trials, transgenic chickpea lines maintained two-fold higher yields compared to non-transgenic Hattrick controls under both mild and severe drought stress. In addition to increased yields, upon stress the transgenic plants produced higher quality grain with reduced tiger striping and increased size. Our results indicate that expression of co-chaperones is a suitable method for the development of elite chickpea varieties that are drought tolerant. The performance of the APSIM model on the basis of average yield was satisfactory with simulated yield. Overall, the results suggest that performance of chickpea genotypes in terms of phenological development and yield under stress conditions could be simulated reasonably well in the glasshouse using the APSIM model. Furthermore, the development of an efficient transformation system provides tremendous potential for the introduction of additional elite traits into chickpea in the future.

POS-THU-128

THE ROLES OF LHX1 AND OTX2 IN MALE GERM CELL DIFFERENTIATION DURING FETAL DEVELOPMENT IN MICE

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Germ cells are the 'ultimate stem cells' in that they are the precursors of the gametes and are, therefore, capable of generating an entirely new organism at fertilisation. In mice, germ cells are set aside from somatic cells at 7.25 days post coitum (dpc), are characterised by continued expression of core pluripotency genes *(Oct4, Sox2, Nanog)* and migrate through the embryo to colonise the developing gonads by 10.5 - 11.5 dpc. Upon entering the developing ovaries, germ cells initiate prophase of meiosis I by 13.5 dpc in response to retinoic acid (RA) in the somatic environment. In contrast, the developing testis degrades RA and produces the male-specific signaling molecules Fgf9 and Nodal such that the germ cells avoid entering meiosis and undergo G0/G1 mitotic arrest by 14.5 dpc. Using expression screening approaches we found that the developmentally conserved homeobox transcription factors Lhx1 and Otx2 are both expressed specifically in male germ cells from 12.5 to 13.5 dpc. Interestingly, others have shown that Lhx1 and Otx2 are expressed in a wide variety of tissues exposed to Fgf and Nodal signaling during fetal development, and have been shown to interact and cooperate in instructing formation of the embryonic head. We examine the effects of germ cell-specific loss of *Lhx1* and/or *Otx2* on expression of core pluripotency genes and previously identified late male germ cell fate markers (*Nanos2* and*Dnmt3L*) to determine the novel roles of these transcription factors in driving male germ cell fate.

POS-FRI-129

EXPLORING THE SIGNALLING MECHANISM OF GLUTAMATE EXCITOTOXICITY IN CULTURED PRIMARY NEURONS BY QUANTITATIVE PROTEOMIC AND PHOSPHOPROTEOMIC APPROACHES

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Excitotoxicity, the overstimulation of ionotropic glutamate receptors is a key process of neuronal loss in acute ischaemic stroke and chronic neurological disorders. However, exactly how neurons die in excitotoxicity still remains unclear. Using stable-isotope dimethyl labelling based quantitative proteomic and phosphoproteomic approaches, we identified at least 80 neuronal proteins showing perturbed expression and 59 phosphoproteins showing significant changes in phosphorylation following 15 min and 4 h of glutamate-induced excitotoxicity. Most of the identified neuronal proteins exhibited reduced expression in excitotoxicity. Signalling network analysis using IPA with these identified protein molecules revealed (i) 14-3-3-mediated signalling, (ii) remodelling of epithelial adherens junctions, (iii) cell cycle including G2/M DNA damage checkpoint regulation, (iv) Myc-mediated apoptosis signalling, (v) PI3K/Akt signalling and (vi) Erk/MAPK signalling as top dysregulated canonical pathways in excitotoxicity. Using similar approach, we also identified significantly dysregulated neuronal proteins and phosphoproteins that are downstream of neurotoxic GluN2B-containing extrasynaptic NMDA receptors. Representative proteomic data were validated by Western blot analysis, also changes in phosphorylation of Mef2c (Ser-222), Mff (Ser-146), Mlf2 (Ser-237) and Stmn1 (Ser-38) were validated by label-free full-scan precursor ions (MS1) quantitation analysis using isotopically labelled synthetic phosphopeptide standards. Our results collectively indicate that inactivation of a number of pro-survival signalling pathways and activation of a series of pro-death signalling pathways cooperate to cause neuronal demise in excitotoxicity. In summary, our findings shed light on the molecular mechanism of excitotoxic neuronal death and identified neuronal proteins are potential targets for the development of neuroprotectants to reduce excitotoxic brain damage in neurological disorders.

POS-WED-130

COMPARATIVE ANALYSIS OF OSMOTINS FROM *TRIPOGON LOLIIFORMIS* AND *ORYZA SATIVA* REVEALED ROLE IN ABIOTIC STRESS TOLERANCE THROUGH SIGNALLING PATHWAYS

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Plant osmotins are a class of pathogen-related proteins that play key roles in both abiotic and biotic stress responses. However, the mechanisms underlying their functions in stress response are not well established due to the lack of information in their interaction partners and pathways. To understand the functions and stress-responsive pathways by which osmotins mediate plant response to stress, an osmotin from the native Australian resurrection grass *Tripogon loliiformis* (*TlOsm*) and two osmotins (*OsOlp1_A* and *OsOlp1_I*) from drought -tolerant (Apo) and -sensitive (IR64) cultivars of the stress sensitive crop, *Oryza sativa* (rice), were compared. Transgenic rice plants expressing the respective osmotins were assessed for enhanced drought tolerance. Additionally, each potential protein partners for each osmotin were assessed by Arabidopsis protein microarray (chip). Evaluation of the transgenic rice suggested functional roles for *TlOsm* and *OsOlp1_A* in enhancing drought tolerance with plants expressing *TlOsm* displaying the highest levels of tolerance. Upon protein array, 270 Arabidopsis proteins significantly interacted with the osmotins, 237 and 239 proteins interacted with OsOlp1_I and OsOlp1_A, while 268 proteins interacted with TlOsm. Interestingly, 11 proteins interacting with TlOsm and OsOlp1_A but not OsOlp1_I, and 21 proteins only interacting with TlOsm were found to be involved in diverse stress-responsive and signalling pathways. GO term enrichment and pathway analysis of osmotin interactors indicated the three most dominant pathways to be Fructose and Mannose metabolism, Glycolysis, and Pentose Phosphate pathway. The differential interactors of these osmotins provide the key mechanisms underlying the function of osmotins in plant stress responses.

POS-THU-131

A MOLECULAR PROBE FOR THE DETECTION OF POLAR LIPIDS IN LIVE CELLS

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Lipids have an important role in many aspects of cell biology, including membrane architecture/compartment formation, intracellular traffic, signalling, hormone regulation, inflammation, energy storage and metabolism. Lipid biology is therefore integrally involved in major human diseases, including metabolic disorders, neurodegenerative diseases, obesity, heart disease, immune disorders and cancers; which commonly display altered lipid transport and metabolism. However, the investigation of these important cellular processes has been limited by the availability of specific tools to visualise lipids in live cells. Here we describe the potential for a molecular probe ReZolve-L1™ to localise to polar lipids in intracellular compartments, containing for example sphingomyelin and phosphatidylethanolamine. In live Drosophila fat body tissue from third instar larvae, ReZolve-L1™ interacted mainly with lipid droplets, including the core region of these organelles. The presence of polar lipids in the core of these lipid droplets was confirmed by Raman mapping and while this was consistent with the distribution of ReZolve-L1™ it did not exclude that this the molecular probe might be detecting other lipid species. In response to complete starvation conditions, ReZolve-L1™ was detected mainly in Atg8-GFP autophagic compartments, and showed reduced staining in the lipid droplets of fat body cells. The induction of autophagy by Tor inhibition also increased ReZolve-L1™ detection in autophagic compartments, whereas Atg9 knock down impaired autophagosome formation and altered the distribution of ReZolve-L1™. Finally, during Drosophila metamorphosis fat body tissues showed increased ReZolve-L1™ staining in autophagic compartments at two hours post puparium formation, when compared to earlier developmental time points. We concluded that ReZolve-L1™ is a new live cell imaging tool, which can be used as a molecular imaging reagent for the detection of polar lipids in different intracellular compartments.

POS-FRI-132

IDENTIFYING THE MECHANISM OF THE MERKEL CELL POLYOMAVIRUS EARLY REGION IN THE INDUCTION OF MERKEL CELL CARCINOMA

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Background Merkel Cell Polyomavirus (MCPvV) is the primary cause of Merkel Cell Carcinoma (MCC), a rare yet highly aggressive type of skin cancer. Tumour suppressor gene INPP4B is significantly downregulated in MCC and other cancers, subsequently, this activates the PI3K/Akt/mTOR signalling pathway, which ultimately leads to uncontrolled cellular proliferation. In this study, we assessed the effect of MCV early region on INPP4B expression and cellular proliferation. We hypothesize that the large T-Antigen (LT) or truncated Large T-antigen (ΔLT) of the MCV early region downregulates INPP4B expression, thus inducing cell proliferation. **Methods** The pLXSN constructs containing either MCPyV full length early region (FL), LT or ΔLT, were transfected into HEK-293, HeLaT cells and primary fibroblasts. The changes in INPP4B expression after transfection were assessed using qPCR. **Result** Transfection of FL, LT or ΔLT in HEK293 and HeLa cells significantly downregulated INPP4B expression. In contrast, the expression of INPP4B expression was upregulated in fibroblasts transfected with FL or LT, and no changes were observed in primary fibroblasts transfected with ΔLT. **Conclusion** The difference in INPP4B expression between the cell lines indicate that INPP4B is already dysregulated in transformed cells. The upregulation of INPP4B expression in primary cells represents natural infection. The absence of INPP4B upregulation in ΔLT suggests that the presence of C-terminus of the MCV early region, which is truncated in ΔLT through truncation, is important in the regulation of INPP4B expression.

POS-WED-133

THE *IN VITRO* AND *IN VIVO* ANTIVIRAL PROPERTIES OF COMBINED MONOTERPENE ALCOHOLS AGAINST WEST NILE VIRUS INFECTION

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West Nile Virus (WNV) is a mosquito-borne flavivirus that can cause neuroinvasive disease in humans and animals for which no therapies are currently available. We studied an established combination of monoterpene alcohols (CMA) derived from Melaleuca alternifolia, against WNV infection. The *in vitro* results show that CMA exhibits virucidal activity, as well as reduces the viral titres and percentage of infected cells. The antiviral mechanism of action of CMA was studied. We found that CMA did not alter the intracellular pH, neither induced apoptosis, but did induce cell cycle arrest in the G0/G1-phase although that was not the antiviral mechanism. Furthermore, we tested CMA *in vivo* using IRF 3-/-/7-/- mice and it was found that CMA treatment significantly delayed morbidity due to WNV infection, reduced the loss of body weight and reduced the viral titres in brain. These findings suggest that CMA could be a therapeutic agent against WNV infection.

POS-THU-134

CATCH ME IF YOU CAN: THE HUNT FOR NOVEL DIAGNOSTIC CANCER BIOMARKERS

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Cancer of the prostate (PCa) and the pancreas (PDAC) are both devastating conditions. In Australia, PCa is the third most common cause of cancer-related deaths. Whilst PDAC has a lower incidence rate, it is often fatal and is the fifth most common cause of cancer-related mortality. Currently used diagnostic biomarkers, including Prostate Specific Antigen (PSA) for PCa and CA 19-9 for PDAC, have shown limited efficacy, with a recent report by the FDA suggesting against the use of PSA in PCa screening. Novel biomarkers, with improved diagnostic efficacy, are therefore required if we are to reduce the burden of this disease. Recently, the homeobox-domain containing gene En2 has been identified in the literature as a potential diagnostic marker for PCa. Our laboratory has identified ectopic En2 expression in PCa as well as in PDAC, which has not been reported previously. This expression has been confirmed both in cancer cell lines and patient biopsies, with an increase in En2 expression identified between benign and matched PCa tumour biopsies from the same patients. Further, we have generated anti-En2 IgY antibodies in a hen model, and verified antibody titres via indirect ELISA. We are currently investigating the kinetic properties of these antibodies to identify their affinity for the En2 protein. Taken together, our results suggest that En2 may be an efficacious biomarker for the detection of PCa and PDAC, and may be useful as part of a diagnostic biomarker panel.

POS-FRI-135

CHARACTERISING THE MUTATIONS INVOLVED IN RESISTANCE TO BIOTIN PROTEIN LIGASE INHIBITORS, A NOVEL CLASS OF ANTIBACTERIAL AGENT TO COMBAT *STAPHYLOCOCCUS AUREUS*

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Staphylococcus aureus is an important clinical pathogen for which current antibiotics are becoming ineffective. The development of new antibiotics is essential to combat these antibiotic resistant infections. One potential target for new antibiotics is the inhibition of the essential metabolic enzyme, biotin protein ligase (BPL). In *S. aureus*, BPL is a bifunctional protein that is both an enzyme and transcriptional repressor of biotin biosynthesis and transport genes. These alternative functions are regulated by the higher order structure of the protein, with the monomer being enzymatic and dimer able to bind DNA. Our approach to target BPL is to design, synthesise and characterise chemical analogues of the reaction intermediate employed by BPL. We have identified a potent antibacterial and pre-clinical candidate, BPL199 (MIC 0.25 - 0.5 µg/ml), with low cytotoxicity observed *in vitro* and *in vivo*. An important consideration during antibiotic development is drug resistance. In this study, bacterial isolates resistant to BPL199 were evolved *in vitro* (n=7) and then characterised using whole genome sequencing. One strain harboured a missense mutation in BPL, D200E, which resides in the dimerisation interface of BPL implying DNA-binding activity may be affected. We demonstrated that the transcriptional repressor activity of D200E was indeed compromised, using a variety of genetic and biochemical techniques including an *Escherichia coli* based reporter system, qPCR and protein cross-linking studies. We propose a novel resistance mechanism whereby the mutation increases the intracellular concentration of biotin that competes with the inhibitor to bind the BPL target.

POS-WED-136

MOLECULAR CLONING DESIGNER SIMULATOR (MCDS): ALL-IN-ONE MOLECULAR CLONING AND GENETIC ENGINEERING DESIGN, SIMULATION & MANAGEMENT SOFTWARE FOR COMPLEX PROJECT MANAGEMENT

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Molecular Cloning Designer Simulator (MCDS) is a powerful new all-in-one cloning and genetic engineering design, simulation and management software platform developed for complex synthetic biology and metabolic engineering projects. In addition to standard functions, it has a number of features that are either unique, or are not found in combination in any one software package: (1) It has a novel interactive flow-chart user interface for complex multi-step processes, allowing an integrated overview of the whole project; (2) It can perform a user-defined workflow of cloning steps in a single execution of the software; (3) It can handle multiple types of genetic recombineering, a technique that is rapidly replacing classical cloning for many applications; (4) it includes experimental information to conveniently guide wet lab work; and (5) it can store results and comments to allow the tracking and management of the whole project in one platform. t is particularly useful for management of complex synthetic biology and metabolic engineering projects. MCDS is freely available from https://mcds.codeplex.com/.