POSTERS

Wednesday - Thursday
Xylan is the second most abundant plant cell wall (PCW) polysaccharide in nature after cellulose. Its complex structures give rise to unique physiochemical properties that influence many biological and physical processes. This makes xylan a vital component for multiple industries including human health and nutrition, renewable biofuel production, food manufacturing and biomedicine. Despite its economic significance, the structure of xylan is less well understood compared to the structure of cellulose. This project aims to carry out functional analysis of key candidate genes from barley and Plantago spp. that are potentially involved in xylan biosynthesis by conducting forward and reverse genetic studies. Our results show that several of the candidates appear to share similar function in different plant species, highlighting their conserved role in xylan biosynthesis. With a better understanding of the xylan biosynthetic pathway, not only can the efficiency of existing industrial processes be improved, it will also enable the engineering of plants with altered PCW structures and/or composition to improve their suite of downstream end uses.

Salinity is one of the major abiotic stresses severely affecting cereal crop growth and yield worldwide. Improvement in salt tolerance of bread wheat is essential to increase food production on agricultural land affected by salinity. In an experiment screening bread wheat diversity lines and two South Australian (SA) bread wheat cultivars Gladus and Scout for salinity tolerance, a Portuguese landrace, Mocho de Espiga Branca (Mocho) showed increased leaf Na+ and similar salinity tolerance as the two SA cultivars under 100 mM NaCl treatment. To determine how Mocho maintained its growth with high leaf Na+ under saline conditions and to identify chromosomal regions associated with its Na+ tissue tolerance phenotype, Mocho was crossed to Gladius. The Mocho x Gladius F2 population were phenotyped for salinity tolerance by switching to CO2 concentrating mechanisms and increasing the vast majority of research on salt stress has focused primarily on cytosolic ion homeostasis with little attention to how salt stress affects chloroplast numbers, function and ion homeostasis in halophytes. Here we highlight how halophytes able to overcome stomatal limitation by switching to CO₂ concentrating mechanisms and increasing the number of chloroplasts per cell under saline conditions. The chlorophyll fluorescence measurements from isolated chloroplasts revealed that halophytes are able to maintain higher relative electron transport rate (RER), maximum quantum yield (Fv/Fm) and steady state fluorescence (Fv) in comparison with glycophytes. Salt-induced changes in ion fluxes from chloroplasts were measured using ion-selective micro-electrode technique. The ion flux measurements revealed that salt stress but not the osmotic stress, induces potassium loss from the chloroplasts. Reducing potassium loss from the chloroplasts found to be more important for maintaining photosynthetic performance. Further, available literature suggests that halophytes accumulate more chloride in chloroplasts than glycophytes and appear to use sodium in functional roles. We propose that the molecular identities of candidate transporters that move sodium, chloride and potassium across chloroplast membranes and discuss how their operation may regulate photochemistry and PSI and II activity in chloroplasts.
**POS-WED-005**

**THE EFFECTS OF HEAT STRESS ON BARLEY FLOWER DEVELOPMENT**

Callens C.1, 2, Tucker M.2, Zhang D.1, 2 and Wilson Z.1

1School of Biosciences, University of Nottingham, Sutton Bonnington Campus, Loughborough, Leicestershire, LE12 5RD, United Kingdom. 2School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae, SA, Australia.

The Poacea, or grasses, include many agriculturally important crops such as rice (Oryza sativa), maize (Zea mays), barley (Hordeum vulgare) and wheat (Triticum aestivum). Unlike dicot plants, the grasses show a highly diverse inflorescence branching architecture. Barley is a widely grown cereal crop that is mainly grown for stock feed, malting and brewing. Abiotic stress is one of the major causes of reduction in crop yield. Many of our cereal crops will need to be redesigned to survive new extremes, with all major climate change models predicting an increase in temperature in the future (Rosenzweig and Parry, 1994). The effects of heat stress on inflorescence architecture and fertility in barley have not been extensively investigated. In this study, we examined three commercial European barley varieties in control and high temperature conditions to investigate the effects on floral development. Using a combination of fertility assays, 3-dimensional modeling, cytology and immunolabelling, we describe the effect of heat stress on floret development and identify cultivar-specific variations in the Poaceae, or grasses, are functionally important in the synthesis of DNA precursors and some amino acids. (DHFR) in particular is a popular target for drug development due to its essential role in the synthesis of DNA precursors and some amino acids. The discovery of Sugars Will Eventually Exported Transporters (SWEETs) in 2010 provided the missing step for which photoassimilates were transported from cells into the apoplasm. Currently, the study of SWEETs is primarily carried out in C₄ species. The focus of this project however, is to elucidate the role of these transporters in C₃ species, more specifically the model plant *Setaria viridis*. Agronomically important crops such as maize and sorghum follow this photosynthetic pathway as well and typically accumulate large amounts of sugar in their sink tissues. Since the discovery of SWEETs it has been found that they can transport a range of sugars such as glucose and sucrose by using heterologous systems such as Xenopus laevis oocytes to determine function. Here, target SWEETs from *S. viridis* are functionally characterized using this system to determine substrate affinity and transport capacity.

**POS-WED-007**

**FUNCTIONAL CHARACTERISATION OF SWEETS IN THE MODEL C₃ SPECIES *SETARIA VIRIDIS***

Chen L.1, Grof C.2, Sharwood R.1, Alonso Cantabrana H.1 and Furbank R.1

1ARC Centre of Excellence, The Australian National University, Canberra. 2The Faculty of Science, The University of Newcastle, Callaghan.

Degradation of purine and pyrimidine nucleotides permits the recycling of phosphate, nitrogen (N) and carbon into central metabolic pools. Purines are degraded to glyoxylate and four molecules of ammonium; the latter can be recycled into amino acids by N-assimilating enzymes. Allantoin, an intermediate of purine catabolism, is a major form for long-distance transport of N in tropical nodulated legumes and it is associated with abiotic stress responses in a range of plant species. Our study revealed that genes in the purine catabolic pathway are highly syntenic among grass genomes. Analysis of Australian wheat genotypes subjected to abiotic and nutrient stress revealed that plants exposed to drought accumulated 2800% and 200% more allantoin in shoot and grains, respectively, than well-watered plants. In contrast, plants exposed to N deficiency had 1400% reduction of allantoin as compared to plants grown under full nutrition. Tissue specific accumulation of allantoin was also found to be transcriptionally regulated by a specific set of purine catabolic enzyme genes including TaXDH1, TaUOX and TaALN. In addition, exogenously supplied xanthine or allantoin recovered N-starved wheat seedlings, as efficient as nitrate. These results indicate that the degradation of purines and more specifically allantoin has a dual role in wheat, as a protective function under drought, and to provide N to support plant growth under N deficiency.

**POS-WED-008**

**CHARACTERISATION OF THE ARABIDOPSIS THALIANA DIHYDROFOLATE REDUCTASE GROUP***

Corral M.G.1, 2, Haywood J.1, 2, Stehl L.H.1, 2, Stubbs K.A.1, Murcha M.W.1, 2 and Mylne J.S.1, 2

1The University of Western Australia, School of Molecular Sciences, 35 Stirling Highway, Crawley, Perth 6009, Australia. 2The ARC Centre of Excellence in Plant Energy Biology, 35 Stirling Highway, Crawley, Perth 6009, Australia. 3The University of Freiburg, Faculty of Biology, Schaufenstrasse 1, 79104 Freiburg, Germany.

The folate synthesis pathway and the key enzyme dihydrofolate reductase (DHFR) in particular is a popular target for drug development due to its essential role in the synthesis of DNA precursors and some amino acids. Despite its importance, little is known about DHFR in plants which, like the off-targeted DHFR from the malaria parasite *Plasmodium*, possesses both a DHFR and thymidylate synthase (TS) domain and so is also likely to be bifunctional. Here we use genetic knockout lines and transient expression of tagged proteins to establish the importance of and subcellular localisation for the proteins encoded by all three *Arabidopsis thaliana* DHFR-TS genes. Genetically, the divergent DHFR-TS3 seemed to be non-functional and is likely to be a pseudogene. A dhfr-ts1 dhfr-ts2 double mutant was embryo-lethal suggesting that DHFR-TS1 and DHFR-TS2 are genetically redundant and required for seed development despite displaying what appeared to be distinct localisation to the cytoplasm and mitochondria respectively. Screening mutated *A. thaliana* seeds for resistance to antifungal DHFR-inhibitor drugs pyrimethamine and cycloguanil identified the causal lesions in DHFR-TS1 and DHFR-TS2 respectively near the predicted substrate binding site. A G137D mutant of DHFR-TS1 conferred resistance to pyrimethamine, cycloguanil, trimethoprim and methotrexate. An A71V mutant of DHFR-TS2 conferred resistance to cycloguanil, partial resistance to trimethoprim and methotrexate, but no resistance to pyrimethamine. These genetic findings were consistent with the biochemical activity of recombinant DHFR-TS1, DHFR-TS2 and mutants thereof. These findings provides a better understanding of plant DHFR-TS and promote the possibility of developing plant-specific inhibitors for plant DHFR as folate synthesis is not a pathway targeted by any current market herbicides.

**POS-WED-009**

**THE EFFECTS OF HEAT STRESS ON BARLEY FLOWER DEVELOPMENT***

Casartelli C.1, Melino V.1, Okamoto M.1, Roessner U.1 and Heuer S.1, 3

1School of Agriculture Food and Wine, Waite Campus, The University of Adelaide, Urrbrae, SA 5064, Australia. 2Metabolomics Australia, School of BioSciences, The University of Melbourne, Parkville, VIC 3010 Australia. 3Plant Biology and Crop Science department, Rothamsted Research, Harpenden, Hertfordshire, AL5 2JG, UK.

The discovery of Sugars Will Eventually Exported Transporters (SWEETs) in 2010 provided the missing step for which photoassimilates were transported from cells into the apoplasm. Currently, the study of SWEETs is primarily carried out in C₄ species. The focus of this project however, is to elucidate the role of these transporters in C₃ species, more specifically the model plant *Setaria viridis*. Agronomically important crops such as maize and sorghum follow this photosynthetic pathway as well and typically accumulate large amounts of sugar in their sink tissues. Since the discovery of SWEETs it has been found that they can transport a range of sugars such as glucose and sucrose by using heterologous systems such as Xenopus laevis oocytes to determine function. Here, target SWEETs from *S. viridis* are functionally characterized using this system to determine substrate affinity and transport capacity.

**POS-WED-009**

**THE EFFECTS OF HEAT STRESS ON BARLEY FLOWER DEVELOPMENT***

Murcha M.W.1, 2 and Mylne J.S.1, 2

1The University of Western Australia, School of Molecular Sciences, 35 Stirling Highway, Crawley, Perth 6009, Australia. 2The ARC Centre of Excellence in Plant Energy Biology, 35 Stirling Highway, Crawley, Perth 6009, Australia. 3The University of Freiburg, Faculty of Biology, Schaufenstrasse 1, 79104 Freiburg, Germany.

The recovery of *Dihydrorotate dehydrogenase* (*DHFR*) in *Arabidopsis thaliana* revealed that this enzyme catalyzes the reduction of dihydrofolate to tetrahydrofolate (THF), in a reaction that is rate-limiting in the folate synthesis pathway. DHFR is a homodimeric protein that contains two NADH-binding sites and a single dihydrofolate binding site. The crystal structure of DHFR reveals a two-fold symmetry and a deep crevice containing the active site. DHFR shows structural homology to the class III dihydrofolate reductase (DHFR) family and is often used as a target for drug development. In this study, we investigated the effects of heat stress on flower development in different cultivars when floral organs are most vulnerable to heat stress. This information will be used to identify and generate barley cultivars that are less susceptible to heat stress at specific stages of floral development.

**POS-WED-009**

**THE EFFECTS OF HEAT STRESS ON BARLEY FLOWER DEVELOPMENT***

Tucker M.2, Zhang D.1, 2 and Wilson Z.1

1School of Agriculture, Food and Wine, Waite Campus, The University of Adelaide, Urrbrae, SA, Australia. 2School of Life Science and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China.
POSTERS

**POS-WED-009**

**ANALYSIS OF PLANTAGO MUCILAGE MUTANTS**

ARC Centre of Excellence in Plant Cell Walls, University of Adelaide, Waite Campus.

This study aimed to identify genes involved in the biosynthesis of polysaccharides by analysing the formation of a specialised secondary plant cell wall: the heteroxylan-rich seed coat mucilage of *Plantago ovata*. Here, we used histological, chromatographic and particle sizing techniques to identify unique phenotypes in three *Plantago* mutants as compared to the wild type, and correlated the phenotypes with transcriptomic changes detected using RNA Sequencing. In one line (star), a mutation of the GT47 *PoKAM1/MUR3/RSA* caused a change in mucilage structure that is likely to be due to the malformation of seed coat cells and a redistribution of mucilage components. Another line (rhonda) showed changes in transcript levels of a subset of three particular GT61 genes may have led to alterations in heteroxylan structure and its interactivity with other polysaccharides in the mucilage. Finally, the mutant aura had an increased amount of cellulose in the mucilage, as detected by fluorescent staining, caused altering patterning of mucilage polysaccharides and was related to a 15-fold upregulation of the *PoCes6A* gene in the developing integument tissues. The results presented here demonstrate that multiple factors can influence the synthesis and properties of mucilage polysaccharides surrounding myxospernumseeds.

**POS-WED-010**

**MECHANISMS OF STOMATAL OPERATION IN QUINOA**

Cuin T.A.1, Hedrich R.2 and Shabala S.1
1University of Tasmania, Hobart, Tas. 2University of Wuerzburg, Germany.

The majority of our crop plants are sensitive to salinity showing major reductions in growth and yield under fairly low levels of salt. However, Quinoa, a highly nutritious pseudo-cereal, is a halophyte. It accumulates huge amounts of NaCl in the shoot, but still survives and even thrives under high salinity. How does Quinoa manage to grow at almost seawater levels? As halophytes can maintain photosynthetic processes even when enormous amounts of sodium are accumulated, they must still be able to maintain significant gas exchange via the stomata without a detrimental loss of water. Hence, the guard cells must be fully functional. How is this achieved? What is so special about the way halophytes operate their stomata to allow the necessary gas exchange? Does sodium play a role? In normal glycophytic plants, potassium is used as an osmoticum for stomatal opening. Sodium, even when available, cannot be used; sodium deposition in a vacuole is a one-way process. Is Quinoa different? Can it use sodium fluxes for stomatal movements, both importing and exporting it from the guard cells to regulate their osmolality? We present X-ray analysis data showing the extent of potassium replacement by sodium in guard cells when Quinoa is subjected to high salinity and demonstrate that sodium levels are lower in these cells at night when the stomata are closed. MIFE measurements reveal the NaCl-induced fluxes of these solutes from these and the surrounding epidermal cells. From this work, we reveal the role that sodium, as a substitute for potassium, plays in Quinoa guard cell function, showing how stomatal operation in halophytes differs from that of glycophytes.

**POS-WED-011**

**CropTiPS: CROP TRANSPORT INFORMATION, PHYSIOLOGY AND SIGNALLING KNOWLEDGEBASE**

David R.1, Hooper C.M.2, Castleden I.R.2, Gillham M.1 and Tyerman S.D.1
1ARC CoE in Plant Energy Biology, University of Adelaide, South Australia, Australia. 2ARC CoE in Plant Energy Biology, University of Western Australia, Western Australia, Australia.

Approximately 25% of all plant genes encode proteins that transport substances across membranes and are involved in diverse cellular reactions. Indeed, in cereal crops like rice, wheat, barley and maize, a large proportion of energy available from photosynthesis is required to transport the nutrients required for growth and grain filling. Although there is a wealth of knowledge in published studies related to crop transport proteins, the information is currently dispersed and unconnected. This makes retrieving relevant information and identifying biological connections a challenging task. We are addressing this issue by constructing a comprehensive knowledgbase of transport proteins in rice, wheat, barley and maize in a database called Crop Transport information, Physiology and Signalling (CropTiPS). CropTiPS integrates experimental transport information from published sources as well as protein metadata and is built using a MySQL relational database platform. The proteins in the database will be aligned to Ensembl identifier system and will be made accessible to users through a web search portal. Experimental information related to a specific transport protein is grouped under three functional categories: 'Transport', 'Physiology & Signalling' and 'Expression' that can be queried through the web portal. The transport information in CropTiPS will help facilitate hypothesis driven research in crop plants and can be investigated for the discovery of associations between stress, transporter expression, nutrient transport, signalling of nutrient status and measured or hypothesized transporter functions, for each of the grain species.

**POS-WED-012**

**THE FUNCTION AND EVOLUTION OF TWO RICE POLLEN ALLERGENS, ORY S 1 AND ORY S 12**

Devis D.D.
University of Adelaide.

Grass allergy is a prevalent disease affecting populations all over the globe, and is caused by allergen proteins in pollen or grains. While vaccine development to alleviate symptoms caused by allergens is heavily researched, little is known about the evolution or function of grass allergen genes. In rice, two multiple gene families that are expressed in pollen, Ory s 1 and Ory s 12, are known to cause an immune response. Using phylogenetic analyses, both these gene families have been shown to have varied evolution, but share the similarity of multiple copies arising after species divergence. Two mutants likes of Ory s1 and Ory s12 were developed through using CRISPR, and the function of Ory s 1 and Ory s 12 is being assessed through phenotype analysis of knockout mutants. Finally, Ory s 1 and Ory s 12 will be expressed and isolated using in order to assess the allergenicity of these proteins. These derivatives may also be used in the development of pollen vaccines.
POS-WED-013

LEAF MATURATION AFFECTS CAROTENOID METABOLISM IN ARABIDOPSIS

Dhami N., Alagoz Y., Tissue D. and Cazzonelli C.
Hawkesbury Institute for the Environment, Western Sydney University, Hawkesbury Campus, Bourke St., Richmond, NSW 2753, AUSTRÁLIA.

Foliar carotenoids contribute to photosynthesis, photoprotection, and phytohormone biosynthesis in plants. Arabidopsis displays characteristic developmental phase change patterns in rosette leaves as the plant grows. The early emerged (juvenile) leaves and laterly emerged (adult) leaves retain their developmental phase identity throughout the lifespan even though both leaf types undergo maturation. We investigated juvenile and adult leaves of Arabidopsis to explore if leaf maturation affects carotenoid accumulation during the developmental phase change. We observed that recently emerged immature leaves comprised significantly higher levels of carotenoids and apocarotenoids in comparison to mature leaf tissues, regardless of their developmental phases. The relative proportions of lutein and violaxanthin decreased whereas that of β-carotene and neoxanthin increased in mature leaves suggesting a metabolic flux reallocation as the leaf maturation progresses. We found significantly higher expression of CCD4 and COB whereas decreased expression of bLYC in mature leaves. The expression level of PSY, CRTISO, and bLCYwas similar in both leaf types. These evidences demonstrate that mature leaf tissues have higher rates of carotenoid degradation due to the higher level of CCDs and shift metabolite flux towards β-chain carotenoids indicating the production and/or requirement of apocarotenoid signals that control leaf maturation. We propose that the age, rather than developmental phase plays a key role in maintaining carotenoid homeostasis and production of apocarotenoids and perhaps immature leaves provide a safer sink to sequester carotenoids. Keywords: carotenoids, apocarotenoids, leaf maturation, developmental phase, Arabidopsis thaliana.

POS-WED-015

INTERACTIONS BETWEEN ELEVATED CO2 AND WARMING IN THE FIELD: THE RESPONSE OF GRAPEVINE PHENOLOGY AND PHYSIOLOGY

Edwards E.J.1, Unwin D.2, Mazza M.2, Mollah M.3 and Treeby M.2
1CSIRO Agriculture & Food, Private Bag 2, Glen Osmond, SA 5064, Australia. 2DEDJTR, crn Koorlong Ave & Eleventh Street, Irymple, VIC 3498, Australia. 3DEDJTR, 110 Natimuk Road, Horsham, VIC 3401, Australia.

Adaptation to climate change is of particular importance to perennial horticulture and other tree crops due to the longevity of plantings. Industry concern in this area to date has largely focused on elevated temperature (eTemp), but elevated atmospheric carbon dioxide (eCO2) is known to not only increase photosynthesis and biomass production in C3 plants, but also commonly reduces tissue nutrient content. Furthermore, in grapevine, the extreme impact of changes in fruit composition (>10 fold range in $value of fruit by weight) means that any effect of a changing climate on the concentration of key secondary metabolites in the fruit will have significant effects on the wine industry. To examine the combined impact of eCO2 and eTemp on mature grapevine plantings in the field, we developed a novel field system (based on open top chambers (OTC)) that incorporates an active heating system, generating 2°C of warming at all times (relative to ambient), and a system that elevates CO2 to 650 ppm. Sixteen OTCs were established, together with non-chamber controls. Warming and eCO2 were imposed in a replicated complete factorial design allowing potential interactions to be determined. The facility was fully operational prior to budburst in 2013 and has run continuously for four seasons. Warming has consistently advanced all stages of phenology, whereas the effect of eCO2 appeared to interact with season. Leaf photosynthesis was invariably higher under eCO2, but eTemp only increased photosynthesis if accompanied by eCO2. Non-structural carbohydrate concentrations were also increased by eCO2, but were lowered by warming irrespective of CO2 concentration. The observed interactions between the treatments and between treatment and season, highlight the need for long-term experiments of this type to discern the true effect of a changing climate on perennial woody crops.

POS-WED-016

PHYSIOLOGICAL TRAITS FOR TOLERANCE TO COMBINED DROUGHT AND HEAT STRESS IN WHEAT

Elhabti A.1, Fleury D.1, Garnett T.1,2 and Tricker P.J.1
1School of Agriculture, Food and Wine, The University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia. 2The Plant Accelerator, Australian Plant Phenomics Facility, PMB1, Glen Osmond, SA 5064, Australia.

Studies predict that drought and heat events will increase in frequency and severity, causing significant crop losses. Despite this, these stresses have rarely been studied together, but their combined effect is known to differ from the effect of each stress singly. The effects of drought and heat cannot be controlled in rainfed wheat growing and new, more tolerant varieties are required to mitigate the adverse effects on productivity. To identify physiological mechanisms involved in plant responses and dissect their genetic basis, eight wheat accessions were selected for their contrasting tolerance to drought and heat. Plants were grown using the Droughtspotter (Phenospex), an automated gravimetric platform with accurate irrigation that allows the control of drought levels and real-time monitoring of transpiration and water use. Drought or combined drought and heat treatments were applied. Plant yield was differentially affected by drought and heat depending on genotype, suggesting that these varieties are a good resource to decipher physiological mechanisms underlying wheat tolerance to these stresses. Interactions between genotype and treatment for total biomass, seed number and water use efficiency were identified under drought, whereas combined drought and heat treatment differentially affected seed weight and vegetative biomass, i.e. the rate of growth and grain filling in heat-stressed spikes. Reactive oxygen species (ROS) contents were measured. Interestingly, the eight varieties contrasted in the response of the ROS scavenging system to the combination of stresses, consistent with recent findings that the ROS scavenging system is a major actor in both signalling and protection from abiotic stress.

POS-THU-014

IDENTIFICATION OF THE PLANT GDP-FUCOSE TRANSPORTER

Ebert B.1,2, Rautengarten C.1, Liu L.2, Pauly M.3, Scheller H.4 and Heazlewood J.1,3
1The University of Melbourne, School of BioSciences, Melbourne, VIC, 3010. 2Joint BioEnergy Institute, Physical Biosciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA 94702, USA. 3Department of Plant and Microbial Biology, University of California, Berkeley, CA 94720, USA.

Glycosylation reactions are fundamental processes of life occurring in all eukaryotes. Many of these essential reactions occur within the Golgi apparatus from cytosolic-derived nucleotide sugar substrates. These activated sugars have to be actively transferred into the Golgi lumen by nucleotide sugar transporters (NSTs). Recently, we have established a yeast protoporospore transport assay coupled to LC-MS designed to assess the transport activities of these NSTs. Using this approach, we identified a transporter capable of transporting UDP-Fuc into the lumen of the Golgi. Due to the newly attributed function of this transporter we named it GDP-Fucose Transporter 1 (GFT1). GFT1 preferentially transports GDP-L-fucose over other nucleotide sugars in vitro while GFT1-silenced plants are almost devoid of L-fucose in cell wall-derived xylglucan and rhamnogalacturan II. Furthermore, those lines display a reduced L-fucose content in N-glycan structures. The decreased L-fucose content of plants with significantly diminished GFT1 expression is accompanied by severe developmental growth defects. Taken together we conclude that GFT1 is the major nucleotide sugar transporter for the import of GDP-L-fucose into the Golgi lumen and is required for proper plant growth and development.
POS-WED-017

LIGHT QUALITY AFFECTS CHLOROPLAST ELECTRON TRANSPORT RATES ESTIMATED FROM CHLOROPHYLL FLUORESCENCE MEASUREMENTS

Evans J.R.1, 2, Von Caemmerer S.1, 2 and Morgan P.B.1
1Division of Plant Sciences, Research School of Biology, ANU. 2ARC Centre of Excellence for Translational Photosynthesis. 3School of Natural Resources, University of Nebraska-Lincoln, Lincoln, NE, USA.

Chlorophyll fluorescence has been used widely to calculate photosynthetic electron transport rates. Portable photosynthesis instruments allow for combined measurements of gas exchange and chlorophyll fluorescence. We analysed the influence of spectral quality of actinic light on chlorophyll fluorescence and the calculated electron transport rate and compared this to photosynthetic rates measured by gas exchange in the absence of photorepiration. In blue actinic light, electron-transport rate calculated from chlorophyll fluorescence overestimated the true rate by nearly a factor of two, whereas there was closer agreement under red light. This was consistent with prediction made with a multilayer leaf model using profiles of light absorption and photosynthetic capacity. Caution is needed when interpreting combined measurements of chlorophyll fluorescence and gas exchange, such as the calculation CO2 partial pressure in leaf chloroplasts.

POS-WED-019

HEAT DAMAGE TO REPRODUCTIVE TISSUE OF OKRA ABELOMOSCHUS ESCULENTUS L. (MOENCH)

Hayamanesh S., Keitel C., Ahmad N., Chatttha T. and Trehovan R.
School of Life and Environmental Sciences, The University of Sydney, Camden, NSW 2570, Australia.

High temperature stress reduces crop yield and is expected to become more frequent with climate change. Sensitivity of reproductive tissues to high temperature has long been recognized and is one of the major reasons for yield reduction. Okra is an important summer vegetable crop and high temperature has been shown to lower its yield. There is, however, limited information on heat damage to okra’s reproductive tissues. We evaluated the changes to male and female organs under high temperature for two consecutive years. Eight genotypes from Pakistan and the World Vegetable Centre were transplanted into control and hot tunnel houses with three replicates per genotype. Temperatures were on average 10°C warmer in the heated tunnel house during the day, and hot tunnel houses with three replicates per genotype. Temperatures were on average 10°C warmer in the heated tunnel house during the day, whereas night temperatures were similar. Under heat stress, the number of fruits decreased dramatically and fruits were exclusively seedless. Light and scanning electron microscopy revealed morphological changes in reproductive tissues. Stained flowers and buds by Safranin-Fast Green showed okra’s male tissue was more vulnerable to heat stress at an early stage of development. Under high temperature, there was a significant reduction in anther numbers, reduced aperture of the stoma and consequently less pollen dispersion and the collapse of pollen structure from a spherical to a flattened shape. Additionally, in vitro pollen germination was dramatically reduced, but stigmas remained receptive as determined by using a Peroxidase esterase indicator, the shape, orientation, curvature and structure of the ovules were similar between treatments. In summary, male reproductive tissues were significantly affected under heat stress, but more detailed analysis is needed to assess the effect of heat stress on the female gametophyte.

POS-WED-018

GENETIC AND MOLECULAR ANALYSIS OF TWO NEW LOCI CONTROLLING FLOWERING IN GARDEN PEA, PISUM SATIVUM

Hasan M., Hecht V., Vander Schoor J.K. and Weller J.
School of Biological Sciences, University of Tasmania, Hobart, Tasmania, Australia.

Flowering is one of the key developmental processes in the plant life cycle and is regulated by different environmental factors and endogenous cues. Isolation and characterization of mutants have been a key research strategy in order to identify genes responsible for flowering in agronomically important legume such as pea, Pismum sativum. This project investigates two novel EMS mutants in the background of cultivated pea line NGB5839 namely late3 and late4 that show extremely late flowering indicating that LATE3 and LATE4 are essential for normal promotion of flowering in pea. Through genetic map and candidate gene analysis, LATE3 and LATE4 genes have been identified as the orthologs of Arabidopsis thaliana Cyclin dependent kinase 8 (CDK8) and Cyclin C (CYCC1) respectively. Besides, we have determined alternative splicing and inferred alternative start codon as the genetic consequences of these mutations. Both CDK8 and CYCC1 are components of the CDK8 module of the eukaryotic mediator complex along with MED12 and MED13, which is known to regulate transcription of many genes. CDK8 module is crucial in maintaining optimum transcription level of such genes in living organisms. Expression analysis of key pea flowering genes such as various FTs have revealed that PsCDK8 and PsCYCC1 mediate expression of these genes which is the potential reason of late flowering phenotype in these mutants. Future experiments will test whether PsCDK8 and PsCYCC1 regulate responses to abiotic factors such as drought, salt stress, light, temperature. The nature of interaction between various components of the pea CDK8 module will also be investigated.

POS-WED-020

TOWARDS UNDERSTANDING THE MOLECULAR CONTROL OF BARLEY SPIKE AND SPIKELET DEVELOPMENT USING A GENOME-EDITING APPROACH

Kuijer H.N.J.1 and Zhang D.1, 2
1University of Adelaide. 2Shanghai Jiao Tong University.

The barley inflorescence is a single spike, where spikelets with single florets develop on the main stem. To understand the molecular control of barley spike and spikelet morphogenesis, we are conducting translational research using the known genes from other model plants such as Arabidopsis and rice. The ABCE model of floral organ development in Arabidopsis was the start of the exploration of the role of MADS box genes in plant inflorescence development. It is a highly conserved gene family and the ABCE model can be applied mostly unchanged to monocots. Some MIKCc type MADS box genes in grasses have additional roles in inflorescence development compared to their Arabidopsis counterparts. I have used agrobacterium mediated barley immature embryo transformation with CRISPR/Cas9 constructs to generate mutants that show a clear phenotype in the first generation. In rice, mutants of one of these MADS box genes have an inflorescence architecture phenotype, while the inflorescence architecture in barley remains unaffected upon knockout of the homologous gene. Expression early in the inflorescence development of these genes is very similar between barley and rice. This could be vestigial expression, which means some of the branching potential is present in barley, but currently, some other component is missing. Since MADS box genes commonly work in tetramers the solution may be found in the search for interaction partners for this gene within the extended gene family. This may lead to opening the toolbox for modification of grass inflorescence architecture.
POSTERS

POS-WED-021

STOMATA ACCLIMATION TO LOW CO2 AND LOW LIGHT IN C4 GRASSES WITH DIFFERENT BIOCHEMICAL SUBTYPES

Israel W.K.1, Watson-Lazowski A.J.1, Chen Z.2 and Ghannoun O.1
1ARC Centre of Excellence for Translational Photosynthesis, Hawkesbury Institute for the Environment, Western Sydney University, Locked Bag 1797, Penrith, NSW 2571, Australia. 2School of Sciences and Health, Western Sydney University, Locked Bag 1797, Penrith, NSW 2571, Australia.

Grass stomata have evolved dynamic response mechanisms to fluctuating conditions thus allowing for relatively high leaf-level water use efficiency (WUE). In this study, we investigated the stomatal responses of grasses with different photosynthetic types (C3 and C4) and C4 biochemical subtypes (NADP-ME, NAD-ME, and PCK) to low CO2 (LC) and low light (LL) conditions. Changes in stomatal morphology (density and aperture) and leaf gas exchange parameters including light transitions (closing and opening half response time (t1/2)) and steady-state stomatal conductance (gₛ) were evaluated. Measured under growth conditions, net photosynthetic rates (Aₛ) were slightly reduced in C4 species acclimated to LC but 50% reduction was observed under LL; while Aₛ of C3 species decreased under LL and LC by 45%. LC increased stomatal conductance (gₛ) by 1.5 fold in PCK and NAD-ME- and 1.9 fold in NADP-ME and C3. LL similarly reduced gₛ in C4 and C3. Measured under high light, gₛ rapidly increased in the C3 and C4 species grown under LC and LL suggesting that acclimation of stomatal conductance has occurred. Increase in stomata density (SD) was observed in C3 and NADP-ME under LC which could explain the higher Aₛ relative to PCK and NAD-ME species. LL reduced SD in the C3, NAD-ME, and PCK species. Stomata aperture (SA) increased at LC, especially in C3 species while smaller SA was observed in the C4 species at LL. Closing t1/2 was unchanged in C4 acclimated under LC while C3 stomata closed 60% more slowly. On the other hand, LL led to 40% faster closure relative to control conditions in both C3 and C4 species. Opening half-time was unchanged in C3 while faster t1/2 was observed in C4 at LC and LL. These results indicate that C4 grass stomata are highly responsive to the environment allowing for higher WUE.

POS-WED-029

PHENOTYPING WHEAT PHOTOSYNTHESIS USING LEAF HYPERSPECTRAL REFLECTANCE

Khan H.A.1, Gaju O.1, Molero G.2, Clarke T.1, Reynolds M.2, Atkin O.K.1, Koerber G.R.1
1Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia. 2International Maize and Wheat Improvement Centre (CIMMYT), Texcoco, CP 56130, Mexico.

Wheat is an important cereal crop contributing to global food security. Growing human population requires a continuous increase in production. While increases in wheat yield gained by modifying harvest index have been fully exploited by plant breeders, improving photosynthesis has the potential to increase wheat yield. Due to a lack of efficient phenotyping methods for photosynthetic traits, it has been hard to explore photosynthetic variation and its genetic regulation. This has prevented the use of photosynthetic traits for crop improvement in wheat. We used ‘leaf hyperspectral reflectance’, a high-throughput phenotyping method, to predict multiple leaf photosynthetic traits in two wheat populations (Seri/Babax, and PSTails) grown at the International Maize and Wheat Improvement Centre (CIMMYT), Mexico. Leaf reflectance spectra were measured on wheat plants at four different growth stages (tiller, booting, anthesis+7 days and grain-filling) from which nitrogen content per unit leaf area (Nₛ), leaf dry mass per unit leaf area, Rubisco capacity per unit area of nitrogen (Vₑ/Nₑ), Vₑ respectively and electron transport rate (Jₑ) were calculated. We observed significant variation for different photosynthetic traits among the genotypes of Seri/Babax and PSTails when measured at different growth stages, but rankings between genotypes were not consistent across different growth stages. The average predicted values for Vₑ, Jₑ, and Vₑ/Nₑ differed among different growth stages. For Seri/Babax and PSTails, predicted values for Vₑ/Nₑ were highest at the tillering stage and lowest at the anthesis+7 stage. Further experiments are being conducted in both Australia and Mexico.

POS-THU-022

ROLE OF TAAALMT1 MALATE-GABA TRANSPORTER IN ALKALINE PH TOLERANCE OF WHEAT

Kamran M., Ramesh S., Bose J., Gillham M. and Tyerman S.D.
Australian Research Council Centre of Excellence in Plant Energy Biology, School of Agriculture, Food and Wine, The University of Adelaide.

Soil alkalinity reduces yield and has been reported as a major problem worldwide, but very little is known about the physiological mechanisms that allow some plants to tolerate alkaline conditions. Previously, it has been reported that Aluminum-activated Malate Transporter proteins (ALMT) play a role in tolerating acidic conditions through carboxylate exudation and these proteins are regulated by gamma-aminobutyric acid (GABA). We have shown from heterologous expression studies that: a) high pH can activate malate efflux and wheat ALMT1 (TaALMT1) carries this flux, b) GABA efflux also occurs with the efflux of malate. On the basis of these findings we hypothesized that TaALMT1 has a role in alkaline soil tolerance. To test this hypothesis, a series of experiments on potted wheat plants were carried out using wheat NIL5; ET8 (Al tolerant, high expression of TaALMT1) and ES8 (Al sensitive, low expression of TaALMT1). In alkaline conditions, a higher concentration of both malate and GABA is found in root exudates from ET8 plants compared with ES8, which appears to decrease the rhizosphere pH more so in ET8 plants. Root biomass was significantly higher in the ET8 plants compared to ES8 plants, and was inhibited by the application of GABA. In control conditions (near neutral pH), exogenous GABA application significantly enhanced transpiration and stomatal conductance in both ET8 and ES8 plants, however in alkaline conditions this difference was not observed. Root expression of TaALMT1 and key genes that are involved in GABA production have also been examined to reveal interesting differences between ET8 and ES8. Our study demonstrates that TaALMT1 plays a role in alkaline soil tolerance by exuding malate and GABA to the rhizosphere and that GABA regulation of TaALMT1 activity results in altered root growth and ultimately leaf photosynthesis.

POS-THU-024

LEAF ANATOMY FOR DROUGHT TOLERANCE IN EUCALYPTUS

Koerber G.R.1, Meyer W.S.1, Cale P.2 and Sun Q.1
1University of Adelaide. 2Australian Landscape Trust, Calperum Station, Renmark.

Whether calculating leaf density (LD, g m⁻²) or leaf mass per area (LMA, g m⁻²), both require leaf thickness (LT, m). LT is the leaf volume to area ratio (LVA, m l⁻²) and it is perpetuated to endeavour to find the significance of the contribution of LD and LVA to LMA and to authenticate the significance of measuring the LMA as a trait for being able to pick plants with advantageous strategies such as allocation of carbon and photosynthetic performance. It is known species with high LMA and LD are from habitats with low water availability. This current research paper wants to flip this on its head and go to leaf density directly in order to recognise the reasons why plants would alter their leaf density. Populations of Eucalyptus mallee, Black Box (E. largiflorens) and River Red Gum (E. camaldulensis) had leaf measurements of leaf density, thickness, weight and area every month for a year. We found leaf density tracked with assimilation calculated from eddy covariance measurements by the TERN Ozflux Calperum tower near Renmark in South Australia. We hypothesise leaf density affects mesophyll conductance and in the future want to record photosynthesis measurements with an infra-red gas exchange system and look at where leaf density is altering within the leaf.
POSTERS

POS-WED-025

SALINITY TOLERANCE IN CHICKPEA IS ASSOCIATED WITH THE ABILITY TO EXCLUDE NA FROM LEAF MESOPHYLL CELLS

Kotula L.1,2, Jimenez J.D.L.C.1, Clode P.L.1 and Colmer T.D.1,2
1UWA School of Agriculture and Environment, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia. 2ARC Industrial Transformation Research Hub on Legumes for Sustainable Agriculture, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia. 3Centre for Microscopy, Characterisation and Analysis, The University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia.

Chickpea is a salt-sensitive species but shows genotypic variation for salt tolerance. We evaluated two contrasting chickpea genotypes: salt-sensitive Rupali and salt-tolerant Genesis836 for ability to exclude Na from leaves. Growth, leaf net photosynthesis, and concentrations of Na, Cl and K in leaflets and various cell types, were measured. Net photosynthetic rates (A) in young leaves of salt stressed (60 mM NaCl) Rupali substantially decreased after 11 days of treatments, whereas Genesis836 maintained A in young leaves for the 21 days of NaCl treatment. Leaflet tissue Na concentration had increased markedly in Rupali, but was low in Genesis836. Cellular (i.e. vacular) concentrations of Na, Cl and K in epidermal and mesophyll cells were measured in frozen-hydrated samples using quantitative X-ray microanalysis. In Rupali, cellular Na concentration was high and similar in all measured cell types of the leaflets (upper epidermis, palisade mesophyll, spongy mesophyll, lower epidermis), whereas in Genesis836 Na was accumulated in epidermal cells but was low in mesophyll cells. Concentrations of Cl and K did not differ between the two genotypes or amongst the cell types. This study indicates that maintenance of photosynthesis and thus salinity tolerance in Genesis836 was associated with an ability to exclude Na from leaflets and in particular from the photosynthetically-active mesophyll cells and compartmentalise Na in epidermal cells.

POS-WED-027

GENETIC CONTROL OF MESOPHYLL CONDUCTANCE IN BARLEY

Li S.1, Dracatos P.2 and Barbour M.M.1
1The University of Sydney, Centre for Carbon Water and Food, School of Life and Environmental Sciences, Camden, NSW 2570, Australia. 2The University of Sydney, Plant Breeding Institute, School of Life and Environmental Sciences, Dobellity, NSW 2570, Australia.

Mesophyll conductance (gm) is now recognized as an important limitation to photosynthesis, as it results in a significant decrease in CO2 concentration from the substomatal airspace to chloroplast stroma. However, little is known about the genetic control of gm. In this study, we used a mapping population of 160 barley lines originated from a cross between Yerong and Franklin. Photosynthetic rate (A), stomatal conductance (gs), leaf intrinsic water-use efficiency (A/gs) and gm were measured in our population and its two parental lines using a coupled leaf gas exchange/13C measurement system. Among the doubled haploid lines, A and gm varied from 12.7 to 41.2 μmol m⁻² s⁻¹ and 0.093 to 0.919 mol m⁻² s⁻¹, respectively. gm varied two-fold, from 0.20 to 0.40 mol m⁻² s⁻¹ bar⁻¹. gm was positively related to A across the genotypes (gm = 0.0075A + 0.1152, R² = 0.5068), but was less closely related to gs (gm = 0.1809gs + 0.22, R² = 0.2694). Quantitative Trait Locus (QTL) analysis was initiated and two QTLs were detected consistently for gm, A and gs. Another promising QTL on chromosome 3 was found for gm and A only. Although preliminary, our results detected regions of genetic control of A and gm. Future work will focus on the genetic localization of these QTLs and comparison with a second barley population.

POS-WED-028

REDUCING THE ENERGY COST OF WHEAT ROOTS

Li X., White R., Richards R., Wasson A. and Ingvorsden C.
CSIRO Agriculture and Food, GPO Box 1700, Canberra, ACT 2601, Australia.

The root system is important in plants for water acquisition, nutrient uptake and anchorage. A large proportion of the carbohydrates produced through daily photosynthesis is required for the production and maintenance of the root system. Our objective is to reduce the energy cost of wheat roots without compromising shoot biomass and yield and eventually to breed wheat lines with more efficient roots to benefit crop production. Little is known about which root traits may reduce energy costs because root systems are complicated and hard to access in soil. This project aims to: 1) assess the importance of various root traits, including nodal root number, root anatomical traits (e.g. cortical cell size) and root respiration - as likely traits to affect the energy cost of wheat roots; 2) screen wheat lines for root traits that may serve an anti-herbivory and UV protection role. However, these questions were explored in detail to understand delayed greening during leaf development in H. prostrata, using metabolomics, and physiological analyses.

POS-WED-026

DELAYED GREENING IN HAKEA PROSTRATA

Kuppusamy T., Lambers H. and Finnegan P.
School of Biological Sciences, UWA, 35 Stirling Highway Crawley, Perth Western Australia 6009.

South-western Australia is a region with soils that are particularly low in phosphorus (P) due to extensive soil weathering and low-P parent materials. Proteaceae growing in south-western Australia are prominent in the region. They have adapted to increase P-acquisition and its efficient assimilation. Hakea prostrata (harsh hakea), a non-mycorrhizal Proteaceae, is endemic to this region and shows P-assimilation and P-use strategies that might be used to improve growth of crops, which rely heavily on P-fertilisers. As such, it has been used as a study species of the region. It was recognised recently that this species also show delayed greening with anthocyanin accumulation during early stages of leaf development. In fact, this trait is common albeit not universal among other Proteaceae. Delayed greening is predominant in tropical forests where it may serve an anti-herbivory and UV protection role. However, a recent study showed that the delayed greening in H. prostrata is reversible when grown with optimal P. This finding suggests that there might be a link to the allocation of resources, especially that of P, during leaf development. This is supported by the delayed accumulation of plastid-localised ribosomal RNA and enzyme activities as well as plastid-targeted lipids during leaf ontogeny. Questions that remain to be addressed include: How does this adaptation work in Proteaceae? What are the components within the developing leaf that control this response? Is delayed greening linked to P-use in Proteaceae? These questions were explored in detail to understand delayed greening during leaf development in H. prostrata, using metabolomics, and physiological analyses.
CELL WALL COMPOSITION AND BIOSYNTHETIC MACHINERY OF THE FUNGAL PATHOGEN FUSARIUM GRAMINEARUM


1ARC Centre of Excellence in Plant Cell Walls, School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Glen Osmond, South Australia 5064, Australia. 2Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Victoria 3086, Australia. 3ARC Centre of Excellence in Plant Energy Biology, Department of Animal, Plant and Soil Science, School of Life Science, La Trobe University, Victoria, 3086, Australia.

The fungus Fusarium graminearum is responsible for the head blight disease in cereals. It is a threat to our food supply due to its negative impact on crop production and produce mycotoxins in infected plant tissues, making them unsuitable for food or feed. The fungal cell wall biosynthetic enzymes are ideal targets for the development of fungicides, however identifying the key genes is challenging without fundamental knowledge of the F. graminearum cell wall composition. Here we present a detailed carbohydrate analysis of the F. graminearum cell wall and a comprehensive expression profile of genes involved in cell wall metabolism and host pathogenesis. Key members of the sugar interconversion pathway have been identified, supporting the presence of sugars found at low levels within the fungal cell wall. Genes that are stably expressed in vitro and in vivo samples provide an overview of the cell wall synthesis machinery, whilst genes that are upregulated across an in vivo time course highlight genes required for pathogenesis and nutrient acquisition. Each gene set contains novel targets for the targeted development of fungicides that either prevent the growth of the fungus by preventing the synthesis of new cell walls or the degradation of the host.

RNA CATABOLISM IN WHEAT: SOURCE OF NUTRIENTS UNDER STRESS?

Melino V.1,2, Casartelli A.2, Rupasinghe T.2, Okamoto M.1, Roessner U.2 and Heuer S.1

1School of Agriculture Food and Wine, Waite Campus, The University of Adelaide, Urrbrae, SA 5064, Australia. 2 - 5 October, 2017, Adelaide, South Australia

Nucleotides, derived from either de novo synthesis or from the breakdown of nucleic acids (DNA and RNA), are essential for plant growth, development and metabolic processes. Ribosomal RNA is targeted to the vacuole in plants to recycle the components (Hillwig et al 2011). This process releases nucleotides that can undergo three fates; intracellular or long-distance transport, salvage or catabolism (Girke et al. 2014). Catabolism of one purine nucleotide releases four molecules of ammonia for subsequent assimilation into amino acids. Bread wheat has a large pool of free purines and pyrimidines in flag leaves (Sawert et al. 1986). Here we ask whether wheat can degrade nucleic acids and nucleotides over five days of nitrogen starvation and whether genotypes differ in their response. Genes involved in degradation, intracellular transport and salvage of N-rich purine nucleotides were identified and their transcriptional profiles examined. A quantitative metabolomics method (HILIC-UHPLC-TQ-MS/MS) was optimised for detection of nucleotides and their catabolites in wheat. The results demonstrate that all wheat genotypes examined can utilise RNA but not DNA, and that rates of RNA degradation were tightly correlated with internal nitrate levels. Purines and their catabolites were reduced in roots but accumulated in the fourth leaf of plants exposed to N starvation suggesting that the urides are transported. In contrast, pyrimidine nucleotides accumulated in roots and the fourth leaf under N starvation suggesting a novel role of pyrimidines in stress signalling. Girke, C., Daumann, M., Niopik-Witz, S., Mohlmann, T (2014) Frontiers in Plant Science 5 (443), 1-12. Hillwig, MS, Contento, AL, Meyer, A, Ebany, D, Bassham, DC, Machlis, GC (2011) Proceedings of the National Academy of Science 108, 1093-1098. Sawert, A., Backer, I., Wagner, K.G. (1988), Plant and Cell Physiology, 29(1):61-65.

PLASMA MEMBRANE AQUAPORINS AS CANDIDATE NON-SELECTIVE CATION CHANNELS IN THE MODEL GRASS SETARIA VIRIDIS

McCaughey S.A., Olu J., Tyerman S.D. and Byrt C.S.

ARC CoE Plant Energy Biology, University of Adelaide, South Australia, Australia.

At the root epithelium, ion influx and efflux is primarily mediated by ion transporters and channels. Despite this, the molecular identity of many of these channels is unknown. However, the recent discovery that an Arabidopsis plasma-membrane localised aquaporin, AQP1P2, functions as a sodium (Na+) permeable water channel in heterologous systems indicates that a subset of aquaporins may be functioning as non-selective ion channels. To determine whether a similar subset of aquaporins in agriculturally relevant species, such as cereal crops, are also permeable to ions, key plasma membrane aquaporins from the model C4 grass species Setaria viridis have been expressed in heterologous systems. Expression of a Setaria viridis plasma membrane aquaporin in the heterologous system Xenopus laevis oocytes induced an ionic conductance, which could be carried either by sodium or rubidium. The effect of signaling molecules associated with abiotic stress, such as cyclic nucleotides, on the water and cation permeability of the Setaria viridis gene of interest. We observed exposure to cyclic nucleotides altered the ionic conductance of oocytes expressing the Setaria viridis plasma membrane aquaporin relative to controls. We hypothesise that exposure to cyclic nucleotides may alter the phosphorylation state of the plasma membrane aquaporins through kinase activation to influence permeability to cations. We believe that further characterisation of the function of putative dual water/ion permeable plant aquaporins will be valuable for informing strategies to improve drought and salinity stress tolerance in crop plants.

CAN H'-PPASE GENES IMPROVE THE GROWTH AND YIELD OF WHEAT (TRITICUM AESTIVUM)?

Menadue D.J., Schilling R.K., Plett D.C. and Roy S.J.

School of Agriculture, Food & Wine, The University of Adelaide, Adelaide, Australia.

To maintain food security, annual cereal production is required to increase to three billion tonnes by 2050. Achieving this yield increase, however, is limited by multiple factors, including abiotic stresses. Proton-pumping pyrophosphatase (H'-PPase) genes have been shown to increase growth and yield under both optimal and stress conditions. Despite three H'-PPase genes (TaVp1, TaVp2 and TaVp3) having been previously identified in wheat, little is known about their function or ability to effect growth and abiotic stress tolerance in wheat. The aim of this study was to identify all H'-PPase genes present in the wheat genome and characterise the role of these genes in plant growth and abiotic stress tolerance. Through bioinformatic analysis of the NRGene wheat reference genome, a novel H'-PPase gene (TaVp4) was identified and homeologous sequences (from the A, B and D genomes) for each TaVp gene were obtained. Expression profiling of the twelve TaVp homeologues revealed significant differences in gene expression between homeologues, tissue types, developmental stages and varieties. These results suggest that the twelve TaVp homologues may have different roles in plant development. Functional differences between the TaVp genes are currently being assessed in yeast. To further investigate the roles of these homeologues, transgenic wheat lines constitutively expressing select TaVp homologues have been generated. Preliminary results suggest TaVp transgenic wheat lines have enhanced growth compared to wild-type. Phenotyping of these lines is currently underway using the high-throughput phenotyping facilities at The Plant Accelerator.
A MICROTUBULE AND CELLULOSE INDEPENDENTLY FORMED UNIFORM WALL LAYER IS ESSENTIAL FOR CONSTRUCTING WALL INGROWTH PAPILLAE IN TRANSFER CELLS

Xia X., Zhang H.M., Patrick J.W. and Offner C.E.
Centre for Plant Science, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia.

Transfer cells are characterized by wall labyrinths with either a flame or reticulate architecture. A literature survey established that reticulate wall ingrowths ubiquitously arise from a modified component of their wall labyrinth, termed the uniform wall layer; a structure absent from flame transfer cells. This finding sparked an investigation of the deposition characteristics and role of the uniform wall layer using a Vicia faba cotyledon culture system. On transfer of cotyledons to culture, their adaxial epidermal cells spontaneously trans-differentiate to a reticulate architecture comparable to their abaxial epidermal transfer cell counterparts formed in planta. Uniform wall layer construction commenced once adaxial epidermal cell expansion had ceased with deposition initiated at the outer periclinal and anticlinal wall junctions to progress inward to ultimately overlay the original outer periclinal wall. A dense ring-like lattice of cellulose microfibrils in the original primary wall was replaced by an overlay of linear cellulose microfibrils sparsely dispersed throughout the polysaccharide matrix to form the uniform wall layer. A re-modelled cortical microtubule array exerted no influence on uniform wall layer formation or on its cellulose microfibril organization. Surprisingly, formation of the uniform wall layer was not dependent upon deposition of a cellulose scaffold. In contrast, uniform wall cells with microfibrils were essential precursors for constructing wall ingrowth papillae. On converging to form wall ingrowth papillae, the cellulose microfibril diameters increased three-fold. This event correlated with up-regulated differential, and transfer-cell specific, expression of VfCesA3B, while transcript levels of other cellulose biosynthetic-related genes linked with primary wall construction were substantially down regulated.

MICROTUBULE AND CELLULOSE INDEPENDENTLY FORMED UNIFORM WALL LAYER IS ESSENTIAL FOR CONSTRUCTING WALL INGROWTH PAPILLAE IN TRANSFER CELLS

Xia X., Zhang H.M., Patrick J.W. and Offner C.E.
Centre for Plant Science, School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia.

Transfer cells are characterized by wall labyrinths with either a flame or reticulate architecture. A literature survey established that reticulate wall ingrowths ubiquitously arise from a modified component of their wall labyrinth, termed the uniform wall layer; a structure absent from flame transfer cells. This finding sparked an investigation of the deposition characteristics and role of the uniform wall layer using a Vicia faba cotyledon culture system. On transfer of cotyledons to culture, their adaxial epidermal cells spontaneously trans-differentiate to a reticulate architecture comparable to their abaxial epidermal transfer cell counterparts formed in planta. Uniform wall layer construction commenced once adaxial epidermal cell expansion had ceased with deposition initiated at the outer periclinal and anticlinal wall junctions to progress inward to ultimately overlay the original outer periclinal wall. A dense ring-like lattice of cellulose microfibrils in the original primary wall was replaced by an overlay of linear cellulose microfibrils sparsely dispersed throughout the polysaccharide matrix to form the uniform wall layer. A re-modelled cortical microtubule array exerted no influence on uniform wall layer formation or on its cellulose microfibril organization. Surprisingly, formation of the uniform wall layer was not dependent upon deposition of a cellulose scaffold. In contrast, uniform wall cells with microfibrils were essential precursors for constructing wall ingrowth papillae. On converging to form wall ingrowth papillae, the cellulose microfibril diameters increased three-fold. This event correlated with up-regulated differential, and transfer-cell specific, expression of VfCesA3B, while transcript levels of other cellulose biosynthetic-related genes linked with primary wall construction were substantially down regulated.
POS-WED-037

IMPROVING YIELD BY OPTIMISING ENERGY USE EFFICIENCY – QTL ANALYSIS IN A LARGE WHEAT RIL POPULATION

Pearson A.1,4, Gillihan M.1,4, Gaju O.1, Wilson P.1, Millar H.1, Borevitz J.3, Akin O.1 and Popgen B.1
1University of Adelaide, ARC Centre of Excellence in Plant Energy Biology, University of Adelaide, Glen Osmond, SA 5064. 2University of Western Australia, ARC Centre of Excellence in Plant Energy Biology, University of Western Australia, Crawley, WA 6009. 3Australian National University, ARC Centre of Excellence in Plant Energy Biology, Australian National University, Canberra, ACT 2601. 4School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Glen Osmond, SA, Australia.

Only 10-15% of the energy captured by plants is allocated to yield with the rest being used in high-cost cellular processes, such as transport of nutrients and respiration. Initial studies performed have shown there is up to two-fold variation in respiration rates of commercial wheat cultivars and heritability of 35%. These results indicate that there is genetic variability in energy use efficiency (EUE) which can be utilised for biomass accumulation and increases in grain yield. In this experiment we have used 1056 lines from an Excilibur x Kukri RIL population to examine the linear phase of growth over a period of time. Imaging was performed at The Plant Accelerator, Adelaide within two temperature controlled Smarthouses containing Lemnatec Scanalyzer 3D imaging stations, capturing images from the top and two sides daily. It was during this period that initial photosynthetic rate measurements were recorded for the 3rd leaf. At the end of imaging photosynthetic rate and respiration were determined on the youngest fully emerged leaf to identify variations in respiratory EUE within the population. QTL analyses are being performed on these traits to identify genotypes and the loci that may provide improvements in EUE and hence improved grain yield for wheat.

POS-WED-038

CELL WALL COMPOSITION OF BLUMERIA GRAMINIS F. SP HORDELI CONIDIA

Pham T.A., Little A., Shirley N.J., Schwerdt J.G., Xing X. and Bulone V. Australian Research Council Centre of Excellence in Plant Cell Walls, School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Glen Osmond, South Australia 5064, Australia.

Blumeria graminis f. sp. hordei (Bgh) is an ectoparasitic obligate biotrophic fungal plant pathogen that exclusively infects barley and causes the disease powdery mildew. It is one of the most economically important diseases to barley as it can cause up to 20-40% losses in yield. All fungi are encompassed by a complex network known as the cell wall. Cell wall synthesis is a fundamentally important process in the growth, survival, and morphogenesis in fungal cells. As the first point of contact between the environment and the fungi the cell wall holds a critical role in determining if the conditions of the environment are hostile or favourable. As the cell wall is important for survival and has many key functions it is an ideal target for cell wall fungicides. While the overall cell wall structure and architecture is conserved across all fungal species, primarily consisting of an intertwined network of chitin, β-1-3-glucans and mannoproteins, it is not the same across all species. Deciphering the structure and composition of the Bgh cell wall will give us a greater understanding of how it works and what aspects to target for inhibition.

POS-WED-039

SEED MUCILAGE MUTANTS ILLUMINATE POLYSACCHARIDE BIOSYNTHESIS

ARC Centre of Excellence in Plant Cell Walls, The University of Adelaide Waite Campus, Urrbrae SA 5064, AUSTRALIA.

When myxospermous seeds are exposed to aqueous environments they extrude a polysaccharide rich gel-like layer, mucilage, which completely envelops the seed and has diverse functions in seed viability. Mucilage is a useful trait to use in the study of polysaccharide biosynthesis and their interactions in gel formation as the extruded mucilage is more accessible than those polysaccharides integrated into the plant cell wall. We are particularly interested in xylan, the second most common plant polysaccharide, and are using Plantago as our model system to uncover the gene expression patterns in xylan biosynthesis. Xylan has a linear β-D-xylosyl backbone decorated with substituent groups that vary in identity, spacing and amount, and from species to species and tissue to tissue. Xylans are a key constituent of cereal grains, particularly wheat, and their variable solubility and fermentability is of particular importance in human health applications. Plantago is a diverse genus comprising over 250 species, their extruded seed mucilage is predominantly composed of heteroxylan. We have used a set of Plantago species with mucilage heterogeneity to track the glycosyltransferase genes responsible for the structural differences of xylan between the species (Phan et al., 2016). Additional to investigating the natural variation between species, we have also developed a mutant Plantago ovata population using gamma irradiation and have screened the seed mucilage of many lines for visible and chemical changes using stains, antibodies, monosaccharide and near-infrared techniques. We have identified exciting mutant mucilage phenotypes and developed a bioinformatic pipeline to find putative causal genomic lesions.

POS-WED-040

EXPLORATION OF THE MITOCHONDRIAL ALTERNATIVE PATHWAY IN WHEAT

Philp-Dutton L.1, Koekemoer F.P.2, Shavrulkov Y.1,3, Day D.A.1,4, Jenkins C.L.D.1 and Soole K.L.1
1School of Biological Sciences, Flinders University, Bedford Park, SA 5042. 2Sensako, Bethlehem 9700, South Africa. 3School of Agriculture, Food and Wine, University of Adelaide, Urrbrae, SA 5064. 4School of Life and Environmental Sciences, The University of Sydney, NSW 2006.

Plants possess two respiratory pathways: the cytochrome pathway (classical electron transport chain) and the alternative pathway (AP), both of which are embedded within the mitochondrial inner membrane. The cytochrome pathway couples the breakdown of organic substrates and the transfer of electrons to proton translocation and ATP synthesis. The AP consists of two main components; alternative oxidases (AOX) and type II NAD(P)H dehydrogenases and unlike the cytochrome pathway, the AP does not contribute to the production of ATP. While the exact role of the AP remains unclear, research has suggested that it may play a role in helping to reduce the production of reactive oxygen species (ROS) by preventing over-reduction of the ETC. This may be important during environmental stresses that induce the production of ROS in cells. Although there has been much investigation into the AP of model plants such as Arabidopsis thaliana, much less is known about the AP in crop plants such as wheat. In this study, a preliminary assessment of AP gene expression of a number of different wheat varieties from different origins has shown a variation in expression levels of the various isoforms of the AP components, even under control growth conditions. Whether this represents a variation in AP protein will be described. Further, how these varieties responded to salinity stress was explored and will be described.
POSTERS

COMPONENTS OF THE NITRATE TRANSPORT AND ASSIMILATORY SYSTEMS OF BARLEY ARE BOTH NITROGEN RESPONSIVE AND DIURNALLY REGULATED

Plett D.C.1, Thomsen H.C.1, Schjorring J.K.2 and Garnett T.P.1
1School of Agriculture, Food & Wine, University of Adelaide. 2Department of Plant and Environmental Sciences, University of Copenhagen.

To explore the relationship between N uptake and assimilation, we studied the diurnal variation of transcripts encoding NO3 uptake transporters and of amino acid concentrations during the vegetative stage of barley plants grown continuously at high or low NO3; or switched between N levels. Previously we established that the high-affinity NO3- uptake system is upregulated when NO3- supply changes over the course of the experiment, indicating its importance is the result of the dynamic responses of the NO3- uptake system. Further investigation of this dramatic, and N supply dependent, diurnal variation in the NO3- uptake system is required to discover regulatory hubs which may be manipulated to improve NO3- uptake and assimilation in plants.

TRANSCRIPTOME ANALYSIS IN ROOTS OF SALT-TOLERANT SOYBEAN GMASLT3-NLINGS USING RNA-SEQUENCING

Qu Y.1, Guan R.2, Berkowitz O.3, Yu L.3, David R.1, Whelan J.2, Wege S.1 and Gilliham M.1
1ARC PEB, School of Agriculture, Food and Wine, University of Adelaide, Glen Osmond, SA, Australia. 2The National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, China. 3ARC PEB, La Trobe University, Bundroo VIC 3083, Australia.

Soybean (Glycine max) is an important crop globally for food and edible oil production. Soybean plants are sensitive to salinity (NaCl), with their yield markedly reduced under saline conditions. 50% reduction at 0·20 dS/m, ~180–200 mM NaCl. GmSALT3 was recently identified, through fine mapping, as a dominant gene underlying a major QTL for salt tolerance in soybean. GmSALT3 encodes a transmembrane protein within the CP2A plant cation/proton exchanger (CHX) family, and is mainly expressed in root phloem and xylem associated cells; the protein was localized to the endoplasmic reticulum. Plants containing a truncated allele (Gmsalt3) are more salt-tolerant than wild type plants. GmSALT3 mRNA levels were upregulated in NILs under saline conditions and demand and this response is regulated by the NRT2 genes. In the present study, barley NRT2 transcripts peaked near mid-day, the peak being significantly higher in low N plants suggesting an important interaction exists between the diurnal and N supply regulatory systems. The NRT2 transcripts adjusted within 6 h of the switch in NO3- supply to the levels of plants grown in the destination NO3- treatment. Nitrate uptake is, in part, regulated by downstream N metabolites. We found the pool of free amino acids in roots showed several distinctive patterns over the diurnal period often with large changes in concentration. The groups of amino acids sharing similar diurnal patterns were distinct between low and high NO3- treatments, however serine had a unique pattern in both treatments. Despite this, the root amino acid levels were unresponsive to N treatments or N supply changes over the course of the experiment, indicating their homeostasis is a result of the dynamic responses of the NO3- uptake system. Further investigation of this dramatic, and N supply dependent, diurnal variation in the NO3- uptake system is required to discover regulatory hubs which may be manipulated to improve NO3- uptake and assimilation in plants.

THE ATSLAH1 AND ATSLAH3 COMPLEX MEDIATES ROOT-TO-SHOOT CHLORIDE TRANSPORT AND IS REGULATED BY MULTIPLE MECHANISMS

Qiu J., Wege S. and Gilliham M.
ARC CoE PEB, University of Adelaide, South Australia, Australia.

AtSLAH1 and AtSLAH3, two slow type anion channel-associated 1 (SLAC1) homologues, are candidates for catalysing root-to-shoot anion transport as both proteins are expressed in Arabidopsis root pericycle and their expression is down-regulated by NaCl and ABA. Previously, we showed that AtSLAH1 expression regulates shoot Cl- accumulation in Arabidopsis, but its expression in X. laevis oocyte led to no detectable anion transport activity. We hypothesized that post-translational modifications were needed to activate AtSLAH1 (Qiu et al., 2016). For instance, AtSLAC1 is positively regulated by phosphorylation by the kinase SnRK2.6 (Geiger et al., 2009). Later, AtSLAH1 was reported to activate Cl- transport through AtSLAH3 (Cubero-Font et al., 2016). Here we explore whether Cl- transport through AtSLAH1:AtSLAH3 is also regulated by phosphorylation. To this end, SnRK2.2, 2.3 and 2.6 were co-expressed with SLAH1:SLAH3 in X. laevis oocyte and anion transport properties (Cl- and NO3-) were examined by Two-Electrode Voltage Clamp (TEVC). Results suggest that SnRK2.2/SnRK2.3 negatively regulates SLAH1:SLAH3, whilst SnRK2.6 does not. To investigate whether SnRK2.2/3.3 can inhibit anion transport through the SLAH1:SLAH3 complex, a predicted phosphorylation site was mutated to create phosphomimic (S179D) and dephosphomimic (S179A) protein versions. Interestingly, SLAH1S179D showed more pronounced inhibition to SLAH1:SLAH3 complex than SLAH1S179T, which still conduct Cl- modestly, whereas SLAH1S179D completely inhibits the complex. These results indicate that this might not be only regulated through phosphorylation event at single target site, as transport was still inhibited. To identify which sites are targeted by SnRks2s in SLAH1 or SLAH3, membrane proteins will be analysed by mass spectrometry. By identifying the upstream regulatory components of SLAH1:SLAH3 complex we expect to further understand the mechanisms plants use to transfer Cl- from root-to-shoot.

UNDERSTANDING THE INTERACTIONS BETWEEN BIOMASS, YIELD AND GRAIN PROTEIN IN HARD AND SOFT WHEAT VARIETIES

Rahimi Eichi V.1, Okamoto M.1, Haefele S.2, Brien C.1, Langridge P.1 and Garnett T.1 3
1University of Adelaide. 2Rothamsted Research, Harpenden, United Kingdom. 3Plant Accelerator, Australian Plant Phenomics Facility.

Most of the efforts for improving nitrogen use efficiency (NUE) in wheat have focussed on hard wheat, but not soft wheat. National variety trial (NVT) data from South Australian sites show that soft wheat lines produce substantially lower grain protein content (GPC) compared to hard wheats. Improving NUE in hard wheat aims to increase nitrogen (N) uptake and/or utilisation efficiency whilst maintaining low GPC. In this context we measured biomass accumulation, relative growth rate (RGR) and N uptake in soft and hard wheats to understand the differences between them. This experiment was designed around non-destructive estimation of biomass using the high throughput phenotyping system at the Plant Accelerator, part of the Australian Plant Phenomics Facility. The automatic imaging system allowed monitoring the biomass and RGR throughout the season. Three levels of N, including 25 (low), 75 (medium) and 150 (high) mg N/kg soil were applied to 3 soft and 3 hard wheat cultivars in 2016. Results showed that differences in projected shoot area (PSA) and RGR between soft and hard wheat genotypes particularly with medium N supply. From medium to high N supply, Spire, a hard wheat cultivar known to produce high GPC, showed the highest increase in PSA compared to other lines.
THE ROLE OF GAMMA AMINOBUTYRIC ACID (GABA) IN ABIOTIC STRESS TOLERANCE IN PLANTS

Ramesh S.A., Kamran M., Sullivan W., Gillham M. and Tyerman S.D.
ARC Centre of Excellence in Plant Energy Biology, School of Agriculture, Food and Wine, Waite Research Institute, University of Adelaide, Glen Osmond, SA 5064 Australia.

GABA is a ubiquitous signaling molecule in animals and plants, however its role in abiotic stress tolerance in plant cells is not well understood. We recently shown that GABA at low micromolar concentrations negatively regulates anion fluxes through Aluminum Activated Malate Transporter family (ALMT) proteins under acid and alkaline conditions and in the range of in vivo concentrations of GABA(1). Further GABA rapidly accumulates in plant cells when they are exposed to low pH, however aluminum exposure reduces endogenous GABA concentrations. The aim of this study was to understand the relationship between anion flux and GABA concentrations in plant cells under abiotic stress. To understand how GABA regulates malate/anion concentrations in plant cells, we examined the effects of agonists and antagonists of mammalian GABA(A) receptors on (Ta)ALMT1 mediated anion flux and endogenous GABA concentrations in wheat genotypes with different levels of expression of TaALMT1, and a transgenic barley overexpressing TaALMT1. A negative linear correlation was observed between the concentration of GABA in the cells and malate efflux under different stress conditions. Interestingly this correlation was lost in TaALMT1 mutants with impaired GABA sensitivity/ regulation. Further, we compared the mutation in GABA concentrations upon aluminum exposure occurs via GABA efflux through TaALMT1 which is very novel as no GABA efflux pathway from cytoplasm to the apoplast has been identified to date. Wild-type TaALMT1 mutants with decreased GABA sensitivity expressed in BY2 cells, Xenopus oocytes and a yeast mutant lacking a GABA transporter, showed that TaALMT1 also transports GABA into the cells. Thus we conclude ALMTs transport both malate anions and GABA and that this transport regulates GABA and malate concentrations in cell cytoplasm and the apoplast. Both these molecules regulate the channel to provide feedback regulation. 1. Ramesh SA, Tyerman SD, et al. Nature Communications. 2015;6.

AVP1, PSTOL1 AND NAS2 - THREE HIGH VALUE GENES FOR HIGHER WHEAT YIELD

Roy S.J.,1 Kalenahalli Y.,2 Sha S.,1 Heuer S.,2 Johnson A.A.T.,1 Gaxiola R.,4 Valluru R.3 and Bailey-Serres J.6
1School of Agriculture, Food & Wine, University of Adelaide, PMB1, Glen Osmond, SA 5064, Australia. 2-Rothamsted Research, Harpenden, AL5 2JQ, UK. 3School of BioSciences, University of Melbourne, Parkville, VIC 3010, Australia. 4School of Life Sciences, Arizona State University, PO Box 874501, Tempe, AZ 85287-2501, USA. 5-Cornell University, Itchaca, New York, 14853, USA. 6-Centre for Plant Cell Biology, Genomics Building 4119A, University of California, Riverside, CA 92521, USA.

Wheat is one of the most widely grown cereal crops, accounting for approximately 20% of daily calories and protein. To feed the world population in 2050 (approximately 9 billion people) a substantial increase in wheat production is required. The International Wheat Yield Partnership has been established to address the challenge of raising wheat yield potential of wheat by 50% in the next 20 years. The genes Vacular Proton Pyrophosphatase 1 (AVP1), Phosphorus Starvation Tolerance 1 (OsPSTOL1) and Nicotianamine Synthase 2 (NAS2) have been shown to improve plant biomass production and grain yield. Over-expression of these genes results in improved biomass production and grain yield in a range of plant species, including cereals (rice, barley, wheat), in optimal growing conditions. Identifying and prioritising the wheat orthologs of these high-value genes provides a real opportunity to produce wheat with significantly improved field performance and higher grain yield. We are using transgenic wheat lines as a proof of concept to determine the optimal combination of the three genes to improve wheat yield; while in parallel, identifying the best natural alleles of the wheat orthologs of these genes for either overexpression or null lines as a proof of concept to determine the optimal combination of the three genes to improve wheat yield. The results indicated that growth at LL caused more significant reduction in rates of linear electron flow at PSI and PSII (ETR(I) and ETR(II)) and cyclic electron flow (CEF), leaf absorbance and rates of enzyme activities than growth at LC for C3, C2 and C4 grass species. The effects of LL + LC treatments were additive, producing more reduction in photosystem activities relative to LL+AC treatment. The greatest reduction in photosynthetic capacity (ETR(I) and CEF) was observed in C2 species and maize (NADP-ME) under LL suggesting that light reactions of these species are more sensitive to changes in irradiance compared to changes in CO2 concentration.

CHARACTERISATION OF TEMPERATURE-DEPENDENT RUBISCO KINETICS IN PLANTS EXHIBITING CRASSULACEAN ACID METABOLISM (CAM)

Rhodes T. and Whitney S.
ARC Centre of Excellence for Translational Photosynthesis, Research School of Biology, The Australian National University, Canberra, ACT, 2601, Australia.

Part of the effort to fortify plants against rising global temperatures and extreme weather events has focused on improving enzymes critical to photosynthesis. In plants, Rubisco is responsible for CO2 assimilation during photosynthesis but exhibits slow, error-prone catalytic properties that are exacerbated at high temperatures. Recent surveys of the temperature response of C3 and C4 Rubisco suggest the kinetics of the enzyme have evolved in response to environmental cues such as temperature (1). Widely recognised examples of plants adapted to hot, dry environments, include those that have evolved Crassulacean Acid Metabolism (CAM). Characterised in part by the closure of stomata during the hottest parts of the day, transpirational cooling is often inaccessible to the fleesy leaves of CAM plants. With elevated leaf temperatures, CAM provides one of the hottest environments within plant for Rubisco catalysis to occur. Indeed, the Rubisco active (a metabolic chaperone needed to maintain Rubisco activity) from the CAM plant Agave tequilana is a highly thermostable enzyme that can maintain high levels of activity at elevated temperatures (2). Here we extend the exploration of the temperature adaptation by photosynthetic enzymes in CAM plants to examine the temperature response of Rubisco from Agave and other important CAM species. This poster will present an update on our progress. 1. Sharwood, R. E., Ghannoum, O., Kapralov, M. V., Gunn, L. H., & Whitney, S. M. (2016). Temperature responses of Rubisco from Paniceae grasses provide opportunities for improving C3 photosynthesis. Nature plants,216186. 2. Shivhare, D., & Mueller-Cajar, O. (2017). Characterization of thermostable CAM Rubisco active reveals a Rubisco interacting surface loop. Plant Physiology, pp-00554.
DISCOVERY OF GENETIC LOCI FOR COMBINED DROUGHT AND HEAT TOLERANCE DURING GRAIN FILLING USING GENOME-WIDE ASSOCIATION IN WHEAT

Schmidt J., Tricker P.J., Garcia M., Eckermann P. and Fleury D.
University of Adelaide, PMB1, Glen Osmond, SA 5064 Australia.

Drought and heat stress often occur simultaneously, particularly during anthesis and grain filling, causing annual yield losses up to 46% in wheat. Aridity and heat events are predicted to increase in severity and frequency in many cropping regions of the world as a consequence of climate change. To ensure wheat’s future productivity, the selection of new, tolerant varieties under both drought and heat stress is required. Genomic association study tests for significant associations of single-nucleotide polymorphisms with certain phenotypes in large, diverse populations. Each study can assess thousands of single-nucleotide polymorphisms at the same time and the identified loci can be used to select for tolerant genotypes. A worldwide collection of 315 diverse wheat genotypes, including landraces, synthetic hexaploids and modern cultivars was analysed using genome-wide association with the aim to identify novel loci for drought and heat tolerance during grain filling. Experiments were carried out in a semi-controlled facility to minimize environmental fluctuations in two successive years. In order to disentangle flowering time effects, genotypes were exposed individually to drought, and drought and heat treatment three days after anthesis. Several quantitative trait loci for drought and heat tolerance have been found for flag leaf water potential, chlorophyll content, spike length, percentage of big seeds, kernel weight, number and weight. These loci have been mapped onto the reference wheat genome to identify putative genes for drought and heat tolerance.

LOCATION, LOCATION, LOCATION: PUTTING SALT SIGNALLING AND ADAPTATION INTO THE CELL-TYPE SPECIFIC CONTEXT

Shahala S.1, Wu H.H.2,3 Shabala L.1, Chen Z.H.4, Bose J.4, Pottosin I.1,5 Mancuso S.1, Zhou M.1, Roessner U.2 and Fuglsang A.T.2
1School of Land and Food, University of Tasmania, Hobart, Tasmania 7001, Australia. 2Department of Botany and Plant Sciences, University of California, Riverside, CA, U.S. 92521. 3School of Science and Health, Western Sydney University, Penrith, NSW 2751, Australia. 4ARC Centre of Excellence in Plant Energy Biology and School of Agriculture, Food, and Wine, University of Adelaide, Glen Osmond, Australia. 5Centro Universitario de Investigaciones Biomedicas, Universidad de Colima, Colima 28045, Mexico. 6Department of Horticulture, University of Florence, Florence 50019, Italy. 7School of BioSciences, University of Melbourne, Victoria 3010, Australia. 8Department of Plant and Environmental Sciences, University of Copenhagen, Copenhagen DK-1871, Denmark.

While the importance of cell-type specificity in plant adaptive responses to salinity is widely accepted, only a limited number of studies have addressed this issue at the functional level. Also elusive remains the nature of putative salt sensors. In this talk, we summarize our current knowledge for the molecular identity of the possible candidates for this role. We then discuss pathways of stress signalling to downstream targets and compare kinetics and specificity of salt stress signalling in various cell types. We show that salinity application to the root apex arrests root growth in a hierarchy- and treatment-specific manner. Although salinity-induced transient net Na+ uptake was about 4-fold higher in the root apex compared with the mature zone, mature root cells accumulated more cytosolic and vacuolar Na+ suggesting that higher sensitivity of apical cells to salt is not related to either enhanced Na+ exclusion or sequestration inside the root. Rather, the above differential sensitivity between two zones originated from a 10-fold difference in K+ retention between the mature zone and the apical region (much poorer in the root apex). The major factors contributing to this poor K+ retention ability are: (1) an intrinsically lower H+-ATPase activity in the root apex, (2) a relative Na+ accumulation and (3) a higher ROS production under NaCl and a larger density of ROS-activated cation currents in the elongation zone. Salinity treatment increased (between 2-5 fold) the content of 10 (out of 25 detected) amino acids in the root apex but not in the mature zone. There were also differences in the levels of organic acids and sugars. The causal link between observed changes in the root metabolic profile and regulation of transporters activity is discussed, and the evidence that meristem cells playing a role of the salt sensor is presented.

UNDERSTANDING THE GENETIC MECHANISMS UNDERLYING MULTIVARIANT MUTANTS TO FACILITATE HYBRID SEED PRODUCTION

Selva C., Tucker M.R., Baumann U. and Whitford R.
School of Agriculture Food and Wine, University of Adelaide, Glen Osmond, 5064, South Australia, Australia.

Wheat (Triticum aestivum) is Australia’s main export crop and is a major source of calories globally. However, new efforts are needed to increase wheat yield and ensure yield stability in light of an increasing world population growth and of the effects of climate change on cropping. Among the different approaches pursued for increasing wheat yield potential, hybrid wheat offers to be a promising solution. The positive effects of heterosis (hybrid vigour) have been extensively documented and are being exploited in economically relevant diploid crops like maize, rice, and sorghum. As promising as it may seem, to date, hybrid wheat is still scarce on the global market compared to other cereals. This is mainly due to the autogamous nature of hexaploid wheat, which ultimately leads to high production costs. This project aims at improving the production of hybrid seeds by redesigning floral architecture. Because of its genetic and physiological characteristics barley (Hordeum vulgare) is used as a model experimental system. Research is conducted on barley multivariants mutants mov1, mov2 and mov5 with the purpose of understanding the genetic mechanisms underlying floral organ specification in cereals. This will help to create a wheat female ideotype for hybrid breeding. In a hybrid breeding scenario, multivariants florets provide the combined advantage of being male-sterile and having an increased chance of successful cross-pollination. This study will provide the necessary tools for lowering the cost of hybrid wheat seed production. Reducing production costs is the first step towards the commercial viability of hybrid wheat breeding.

GENETIC ANALYSIS OF ROOT TRAITS ASSOCIATED WITH SALT-TOLERANCE IN A BARLEY MAPPING POPULATION

Sheiden M.C.1, Brien C.2,3 and Tyerman S.D.1
1ARC Centre of Excellence in Plant Energy Biology, School of Agriculture, Food and Wine, University of Adelaide, Glen Osmond, SA, Australia. 2Phenomics and Bioinformatics Research Centre, The University of South Australia, Adelaide, SA, Australia. 3Australian Plant Phenomics Facility, The Plant Accelerator, University of Adelaide, Glen Osmond, SA, Australia.

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 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 can maintain root elongation at salt concentrations that inhibit root growth; an adaptive mechanism to ensure seedling establishment and maintain water and nutrient uptake. We aim to identify the key genes and pathways in barley roots that are involved in both perceiving osmotic changes in the soil and influencing root elongation, ultimately to increase salinity tolerance in crops. Barley cv. Clipper (malting barley) and Sahara (North African landrace 3771), have previously been shown to have a contrasting root growth phenotype in response to the early phase of salinity stress. To elucidate the genetic basis for these mechanisms, a Clipper x Sahara double haploid mapping population has been screened for shoot and root phenotypic traits in response to salt stress. We are currently conducting a genetic analysis of the mapping population using Quantitative Trait Loci analysis 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.

GENETIC ANALYSIS OF ROOT TRAITS ASSOCIATED WITH SALT-TOLERANCE IN A BARLEY MAPPING POPULATION

Shelden M.C.1, Brien C.2-3 and Tyerman S.D.1
1ARC Centre of Excellence in Plant Energy Biology, School of Agriculture, Food and Wine, University of Adelaide, Glen Osmond, SA, Australia. 2Phenomics and Bioinformatics Research Centre, The University of South Australia, Adelaide, SA, Australia. 3Australian Plant Phenomics Facility, The Plant Accelerator, University of Adelaide, Glen Osmond, SA, Australia.

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 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 can maintain root elongation at salt concentrations that inhibit root growth; an adaptive mechanism to ensure seedling establishment and maintain water and nutrient uptake. We aim to identify the key genes and pathways in barley roots that are involved in both perceiving osmotic changes in the soil and influencing root elongation, ultimately to increase salinity tolerance in crops. Barley cv. Clipper (malting barley) and Sahara (North African landrace 3771), have previously been shown to have a contrasting root growth phenotype in response to the early phase of salinity stress. To elucidate the genetic basis for these mechanisms, a Clipper x Sahara double haploid mapping population has been screened for shoot and root phenotypic traits in response to salt stress. We are currently conducting a genetic analysis of the mapping population using Quantitative Trait Loci analysis 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.
Our research demonstrates RMD mediated F-actin plays a crucial role in defective shoot gravitropism and amyloplast movement. In summary, similar to rmd mutants, OsPIL16 overexpression lines also exhibit more sensitivity to LatB treatment (inhibitor of actin polymerization). This suggests that RMD transcriptional level could be directly repressed by light both in light and diurnal cycle, furthermore, dual-LUC assay demonstrate novel role of F-actin in photomorphogenesis, which mediated by RMD protein. Corresponding to the light-dependent phenotype, rmd mutants were found to be closely related to bunch compactness. It is concluded that canopy management practices can be applied to manipulate reproductive performance through different mechanisms. Besides influencing yield components, canopy management also impacts bunch morphology, which is relevant to disease management.

**POS-WED-054**

**EXPRESSION PROFILING OF THE AMMONIUM TRANSPORTER FAMILIES (AMT AND AMF) IN SOYBEAN NODULES**

Uddin M.K., Wen Z. and Kaiser B.N.

The University of Sydney.

Ammonium (NH4+), a major nitrogen (N) source for plant growth and development. Among leguminous plants, soybean (Glycine max L.) is an important global crop, grown as a rotation legume predominantly for human oil consumption and animal feed. Soybean is traditionally associated with high atmospheric N fixation capacity. In this study, we performed expression profiling and phylogenetic analysis of ammonium transport (AMT1, AMT2) and ammonium facilitator (AMF1) genes in soybean nodules. The soybean genome contains at least putative 14 AMTs (5 AMT1s and 9 AMT2s) and 5 AMFs, a large number relative to that reported in the model plant Arabidopsis thaliana and other model-legumes like Medicago truncatula and Lotus japonicus. Using online soybean RNA-seq transcriptome data sets, we identified 8 highly expressed NH4+ transporters in nodules (GmAMT1:2, GmAMT1:3, GmAMT1:6, GmAMT2:1, GmAMT2:2, GmAMF1, GmAMF4 and GmAMF5). qPCR primers were designed to analyse transcript abundance in rhizobia inoculated plants grown in the absence of external N. Transcript levels of GmAMF3, GmAMF5 and GmAMT1:6 gradually increased after 15 day post inoculation (dpi), and were highest at around 35 dpi while GmAMT2:1 and GmAMT2:2 showed highest transcript levels at around 15 dpi. GmAMT1:2 transcript levels increased initially but decreased after 10 dpi but increased again after 30 dpi. GmAMT1:3 showed the highest transcript levels at 10 and 30 dpi. Transcripts of GmAMF4 were at their highest levels at around 30 dpi.

The initial transcriptomic information from this study is helping to define the functional roles of NH4+ transport systems that operate in soybean nodules to capture and redistribute ammonium to the plant.
In many plant species, transport from source to sink tissues includes a requisite apoplastic step. Transfer cells (TCs) form at these bottlenecks for nutrient transfer, and are discernible by a morphologically unique, intricate meshwork of cell wall material polarized to sites of apoplastic nutrient flow, termed a wall labyrinth (WL). A dramatic increase in plasma membrane surface area, determined by the degree of WL deposition, confers the enhanced nutrient transport capability of TCs. The experimental impasse presented by the low abundance of TCs and their predominant location deep within tissues in vivo has been circumvented by exploiting the capacity to induce TC trans-differentiation in the epidermal cells of broad bean (Vicia faba) cotyledons in vitro. Using this model system, key inductive chemical signals along with the changes in gene expression that accompany the temporal pattern of WL formation have been elucidated (Andriunas et al., 2013). Despite this substantial body of knowledge, and the unique structure and function of the TC WL, it remains unclear whether the TC WL is compositionally specialized. Using a suite of plant cell wall monoclonal antibodies in conjunction with confocal and transmission electron microscopy, we show that the distribution of numerous pectic and hemicellulosic cell wall epitopes differ significantly between the WL and adjacent cell wall matrices. With this information, we propose a new model for the cell wall biosynthetic mechanisms that underlie wall labyrinth construction and discuss how the spatial distribution of constituent polysaccharides potentially influence wall labyrinth structure and consequently apoplastic transport rates.

**POS-WED-058**

**USING BIFC TO EXAMINE PLANT NATRIURETIC PEPTIDE INTERACTION WITH CATALASE 2**

**Wheeler J.I.1,2, Turek I.3,4, Irving H.R.1 and Gehring C.3,5**

1Monash Institute of Pharmaceutical Sciences, Monash University, Melbourne, VIC 3052 Australia. 2La Trobe University, Dept Animal, Plant and Soil Sciences, AgriBio, Melbourne, VIC, Australia. 3Division of Biological and Environmental Science and Engineering, King Abdullah University of Science and Technology, Thuwal, Kingdom of Saudi Arabia. 4Leibniz Institute of Plant Biochemistry, Leipzig, Germany. 5Cambridge Centre for Proteomics, Department of Biochemistry, University of Cambridge, Cambridge, United Kingdom.

The Arabidopsis thaliana PLANT NATRIURETIC PEPTIDE (APNPA) has been implicated in a range of plant processes including ABA dependent stomatal regulation and systemic acquired resistance immune responses. Previously we have shown AIPNP-A induces the production of reactive oxygen species in suspension-cultured A. thaliana (Col-0) cells. H2O2 is an important signalling molecule regulating plant growth and stress responses. CATALASE 2 (Cat2) catalyses the decomposition of H2O2 in photosynthesis tissues. Cat2 activity is reduced in atpnpa mutants suggesting AIPNP-A may enhance the enzymatic activity of the Cat2 protein. Here we use BiFC to show the interaction between AIPNP and Cat2 in infiltrated tobacco leaves. Interaction between AIPNP-A and Cat2 might indicate that Cat2 may mediate the previously observed activity of AIPNP-A in counteracting the ABA-dependent stomatal closure.

**POS-WED-059**

**IDENTIFICATION OF MOLECULAR CUES INFLUENCING OVULE DEVELOPMENT IN BARLEY**

**Wilkinson L.G.1,2, Houston K.1,2, Byrt C.S.1,2, Burton R.A.1,2 and Tucker M.R.1,2**

1Australian Research Council Centre of Excellence in Plant Cell Walls, Adelaide, Australia. 2School of Agriculture, Food and Wine, University of Adelaide, Australia. 3The James Hutton Institute, Dundee, United Kingdom.

Seed development in plants is regulated by successful development of male and female reproductive tissues. A key component of reproductive development is establishment of a germline, leading to production of gametes. In monocot cereal crops, female gametogenesis takes place in a tissue called the nucellus, a somatic cell mass located at the distal tip of the ovule, surrounded by a ring of integuments. The nucellus performs a similar role between species; it gives rise to the female gametophyte, provides a protective physical barrier, releases signals regulating female gametophyte progression, and after fertilization it acts as a nutrient transfer tissue to support downstream stages of seed development. Genetic and molecular understanding of female reproductive development in cereals is far from complete, and even in the case of dicots such as Arabidopsis the molecular identity of the nucellus remains unclear. This project aims to characterise genetic and molecular regulatory pathways underlying barley ovule development, with a particular focus on the nucellus and gametophyte. A genome wide association study (GWAS) on a panel of 165 two-row spring barley cultivars revealed four loci that influence morphological variance. Transcriptional profiling of developing barley ovaries revealed dynamic gene expression changes in throughout female gametophyte development. Alignment of genetic, physical and transcriptional datasets enabled identification of specific candidate genes for characterization of expression and function during ovule development. This data provides a foundation for future studies of ovule development and their contribution to downstream seed size, morphology and composition in cereal crops.
POSTERS

POS-WED-061
ICE ACTIVE PROTEINS IN NEW ZEALAND CHIONOCHLOA SPECIES
Xiong H., Marshall C., Wharton D. and Lord J.
University of Otago, New Zealand.

Unlike fishes and insects secret cryoprotectants to depress the freezing point of body fluid, overwinter plants produce ice active agents with much lower thermal hysteresis but higher ability of inhibiting ice recrystallization. Plants also minimize the damage from freezing by triggering ice formation at high sub-zero temperatures with ice nucleation agents. Chionochloa are tussocks mainly endemic to New Zealand and dominate the alpine grasslands. Previous studies indicated significant ice nucleation activity (INA) (~5°C), in two alpine Chionochloa species, C. macra and C. rigida, but provided no more information about other ice activities in this genus. We investigated 17 of 34 Chionochloa species and found seasonal variations of ice activities in most collections with winter collections showed the highest activities. We showed here that intrinsic proteins are essential in maintaining all three types of ice activity, which showed different responses to treatments including heat, pH, high salt, protease K, lysosome, reducing and oxidizing agents. Ice affinity purification indicated the presence of antifreeze proteins in C. macra, which was confirmed by mass spectrometry and transcriptome analysis from overwinter C. macra. Amino acid alignment analysis showed highly conserved ice-binding domains in the recrystallization inhibition proteins (RIPs) among C. macra and grass species including Lolium perenne and Deschampsia antarctica. Phylogenetic analysis of cold stress related genes, IAPs, C-repeat binding factors and fructosytransferase, in C. macra and other grass species indicated these genes from C. macra did not split with the core Pooidae family compared with genes from Brachypodium distachyon. Protein expression confirmed ice activities in codon optimized ice active genes in C. macra.

POS-WED-062
STRUCTURAL VARIATIONS IN WHEAT HKT1;5 UNDERPIN DIFFERENCES IN NA+ TRANSPORT CAPACITY
Xu B.1, Waters S.1, Byrt C.S.1,2, Piett D.2, Tyerman S.D.1,3, Tester M.2, Munns R.1,3, Hrmova M.1,3 and Gilliham M.1,3.
1Australian Research Council Centre of Excellence in Plant Energy Biology, University of Adelaide, Waite Research Precinct, Glen Osmond, South Australia 5064, Australia. 2School of Agriculture, Food and Wine, and Waite Research Institute, University of Adelaide, Waite Research Precinct, Glen Osmond, South Australia 5064, Australia. 3Center for Desert Agriculture, Division of Biological and Environmental Sciences and Engineering, 4700 King Abdullah University of Science and Technology, Thuwal 23955-6900, Kingdom of Saudi Arabia. 4School of Agriculture and Environment, and ARC Centre of Excellence in Plant Energy Biology, University of Western Australia, Crawley 6009, Australia.

An important trait associated with the salt tolerance of wheat is the exclusion of sodium ions (Na+) from the shoot. The sodium transporters TmHKT1;5-A and TahKT1;5-D, from Triticum monococcum (Tm) and Triticum aestivum (Ta), are encoded by genes underlying the major shoot Na+-exclusion loci Nax1 and Kna1, respectively. Here, using heterologous expression we show that the affinity (Kₐ) for the Na+ transport of TmHKT1;5-A, at 2.66 mM, is higher than that of TahKT1;5-D at 7.50 mM. Through 3D structural modelling we identify residues – D___/a gap and D___/G/I/2 that contribute to this property. We identify 4 additional mutations in amino acid residues that inhibit the transport activity of TmHKT1;5-A, which are predicted to be the result of an occlusion of the pore. We propose that the underlying transport properties and localisation of TmHKT1;5-A and TahKT1;5-D contribute to their unique ability to improve Na+ exclusion in wheat that leads to an improved salinity tolerance in the field. Based on our data, we define Na+ transport by HKT proteins within root vasculature tissues as a ‘gatekeeper’ process that secures shoot Na+-exclusion and underpins recent improvements of crop plant salt tolerance.

POS-WED-063
A GLUTAMYL-TRNA SYNTHETASE REGULATES ANther CELL DIVISION AND PATTERNING IN RICE
Yang X.J., Li G., Song Y. and Zhang D.B.
School of Agriculture, Food and Wine, University of Adelaide, South Australia 5064, Australia.

Anther development distinguishes itself from life cycle of higher plants by strictly controlling the cell lineage and raising germinal cells within a specific niche. Maintaining an appropriate physiological status is essential to establish organ pattern and function. The regulatory components of homeostasis maintenance during early anther development is however largely unknown. Here we report OsERS1, a glutamyl-tRNA synthetase from rice (Oryza sativa L.) which functions in cell division and patterning in anther. We confirm the catalytic activity of OsERS1, enhanced by cofactor OsARC (aminoacyl-tRNA synthetase co-factor). osers1 anther exhibits impaired male fertility, excess vitality of cell division and disorganized cell layers. Metabolically, osers1 has more active mitochondria and rewired amino acids-centric metabolism. Further, disorder of redox status in osers1 anther contributes to abnormality and treatment with hydrogen peroxide in wild-type anther perfectly mimics osers1 mutant-characterized phenotypes. Taken together, our results demonstrate that OsERS1 occupies a key position in modulating the metabolism network and cellular redox status to control early anther development in rice.

POS-WED-064
HIGH-THROUGHPUT NON-INVASIVE PHENOTYPING REVEALS SALINITY TOLERANCE IN AUSTRALIAN WILD RICE SPECIES DURING SEEDLING GROWTH
Yachie Y.,1 Brien C.J.3,4, Jewell N.D.3, Roberts T.H.1 and Atwell B.J.2
1School of Life and Environmental Sciences, University of Sydney. 2Department of Biological Sciences, Macquarie University. 3Australian Plant Phenomics Facility, University of Adelaide. 4Australian Research Council Centre of Excellence in Plant Energy Biology, University of Western Australia.

While cultivated rice (Oryza sativa) provides the primary source of nutrition for more than one-third of the world’s population, relatively little use has been made of the vast genetic diversity found in the 23 wild species of Oryza worldwide in breeding for resistance to abiotic stresses. Increase in soil salinity levels is becoming a major cause of crop yield losses worldwide. Salinity limits rice growth and yield, particularly in coastal growing areas. Modern rice cultivars are highly sensitive to salinity, especially during early vegetative and reproductive stages. In an effort to address this problem, we evaluated accessions of O. australiansis and O. meridionalis endemic to the savannah of northern Australia. Plants were assessed at the seedling stage at sodium chloride concentrations up to 80 mM. Multiple accessions were screened for resistance with O. sativa genotypes ranging from salt sensitive (IR29) to tolerant (Pokkali). An initial greenhouse-based screening revealed substantial salt tolerance in some but not all native accessions. To validate this screen, non-destructive image-based phenotyping was performed at the Plant Accelerator, an Australian national plant phenotyping facility. Reductions in projected shoot area, sodium/potassium ratio and photosynthetic parameters were less marked in the salt-tolerant accessions even at 100 mM NaCl, while significant impacts of concentrations of salt as low as 40 mM were observed in the sensitive accessions. In addition, leaf senescence was alleviated in salt-sensitive lines at 40 mM salt, while milder symptoms were observed in the tolerant genotypes and were only apparent at higher salt treatments. These results indicate that the Australian wild Oryza species exhibit considerable tolerability to salt levels that damage O. sativa and should be investigated as a source of salt tolerance in breeding programs.
Chloroplast functions as an initial sensor of environments and its status is a key regulator in ABA induced plant defenses, for instance, stomatal closure under drought. 3-phosphoadenosine 5-phosphate (PAP) is a retrograde signal which enhances plant drought tolerance by inducing stomatal closure. We found (PAP) signal functions more broadly in guard cells of a range of evolutionarily important plant species. A combination of genetics, biochemical and electrophysiology approaches show that chloroplast communication via PAP forms a novel secondary pathway in abscisic acid (ABA)-mediated stomatal closure during drought stress. Transcriptome of isolated Arabidopsis mesophyll and guard cells revealed that PAP differentially regulates key ABA signaling components in the wild type, a PAP accumulator - altered expression of APX2 (axl8), drought-sensitive Open Stomata 1 (ost1-2) and ost1-2/axl8 double mutant. Reactive oxygen species, nitric oxide, ion fluxes, and ion channels were found to be differentially regulated in guard cells of the four Arabidopsis lines. In summary, PAP may function at downstream of the core ABA reception complex - regulatory component of ABA receptors (RCARs), ABA insensitive 1 (ABI1) and OST1, therefore bypass those essential components for stomatal closure. We demonstrate how a chloroplast signal can directly intersect with and fine-tune ABA signaling for drought response in Arabidopsis.

Mollusc shells have excellent combinations of strength and stiffness. This is because calcareous shells are composites of inorganic matrix with organic materials, which are spatially ordered in an exquisitely controlled architecture. It is well known that shell macromolecules are a minor (~ 5 wt%) albeit vital component in shell-bearing animals: they regulate the precipitation of mineral, and select the polymorphs of calcium carbonate - calcite and/or aragonite. In addition, it is believed they determine the shell microstructure. The molecular structure that leads to the functional properties of calcareous shell, however, is far from being fully understood. We used solid state-NMR complemented with other analytical techniques to explore the composition of the organic part of bivalve shells and examine its interaction (if any) with the inorganic part of the shells. The biorganic content consists mainly of proteins and consistently shows the chemical shifts of silk-like fibres i.e. alanine and glycine residues. This composition is strikingly similar to the constituents of spider dragline silk but does not conform to the accepted view that the biorganic component in shells consists of well-arranged insoluble chitin [1]. Overall, the present work depicts that silk-like fibres exist in B-sheet conformations, comprising hydrogen bonded nanocrystals structure and semi-amorphous fibrillar structure, are a significant constituent in shell-bearing molluscs, which has been completely unknown before. [1] Y. Levi-Kalisman, G. Falini, L. Addadi, S. Weiner, Journal of structural biology 135 (2001) 8-17.

**POS-WED-065**

**RETROGRADE SIGNAL SAL1/PAP PATHWAY IS EVOLUTIONARY CONSERVED FOR STOMATAL REGULATION IN PLANTS**

Zhao C.1, Wang Y.1, Chan K.X.2, Haigh T.1, Holford P.1, Pogson B.2 and Chen Z.1

1School of Science and Health, Western Sydney University, Penrith, NSW 2751, Australia. 2College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China. 3ARC Centre of Excellence in plant Energy Biology, Research School of Biology, The Australian National University, 134 Linneaus Way, Acton ACT 2601, Australia.

**POS-WED-067**

THE ORGANIC MATRIX IN MOLLUSC SHELLS WITH STRUCTURAL ANALOGUES TO SPIDER SILK

Agbaje O.B.A.1, Shir I.B.2, Zax D.1, Schmidt A.1 and Jacob D.E.1

1Department of Earth and Planetary Sciences, Macquarie University, NSW 2109 Australia. 2Schulich Faculty of Chemistry and Russell Berrie Nanotechnology Institute Technion-Israel Institute of Technology, Technion City, Haifa 32000, Israel. 3Department of Chemistry, Cornell University, Ithaca, New York 14853 USA.

**POS-WED-068**

ALLEVIAION OF ABIOTIC STRESS IN CANOLA USING GENETIC ENGINEERING

Alahakoon A.Y.1, Tongson E.J.1, Chye M.L.3, Golz J.F.2, Russell D.A.1 and Taylor P.W.J.1

1Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC, Australia. 2School of Biosciences, The University of Melbourne, Parkville, VIC, Australia. 3School of Biological Sciences, The University of Hong Kong, Pokfulam, Hong Kong.

Brassica napus (canola) is the second largest oilseed crop grown worldwide with the annual production in Australia being around three million metric tons. The canola industry is globally affected by abiotic stress conditions such as drought, high salinity, frost and biotic stresses imposed by fungi, bacteria and insects. There is a pressing need for crop trait improvement in canola to better withstand these stress conditions. In this regard, genetic engineering could be a useful tool to introduce novel genes that have the potential to alleviate such stresses. The Acyl-CoA-Binding Protein (ACBP) family is thought to be involved in intracellular acyl-CoA ester transport and has been implicated in stress mediation in many organisms. Frost tolerant rapid-cycling B. napus and canola cv. Westar were developed by introducing the Arabidopsis thaliana ACBP6 cDNA using Agrobacterium-mediated gene transformation. Transgenic Brassica lines at T0 progeny were tested for frost tolerance in cold-acclimated and non-acclimated conditions in an upright fan-forced freezer with an electronic temperature controller. Electrolyte leakage, which arises from damage to biological membranes during freezing, was measured and found to be lower in the transgenic lines than in the wild type in non-acclimated conditions. Seed survival was higher in the transgenic lines after short-term and prolonged freezing in non-acclimated conditions. These findings indicate that the overexpression of ACBP6 is potentially useful in making canola crops more tolerant to frost in the field situations. This remains to be tested.

**POS-WED-069**

**DIFFERENTIAL PLASTICITY OF ABOVE- AND BELOW-GROUND TRAITS AMONG SIX PRUNUS TAXA UNDER SHORT-TERM VERSUS PROLONGED SEVERE WATER STRESS**

Zhou S.X., Walker R. and Edwards E.

CSIRO Agriculture and Food, Locked Bag 2, Glen Osmond, SA 5064, Australia.

Soil water stress can cause negative impacts on plant survival and growth. The drought-response relationships of above- and below-ground plant traits are largely unknown. A glasshouse experiment involving six Prunus taxa whose above- and below-ground traits were measured after short-term and prolonged watering treatments (100% field capacity as control and 33% of evapotranspiration of control plants as severe water stress treatment) was conducted to test relevant hypotheses. We found that the degree of plasticity of above- and below-ground plant traits is dependent on the taxa and the duration of water stress. We also found a large variation among the drought responses of fine-root morphological traits. These results indicate that drought-responses of above- and below-ground plant traits can differ across species and time scales, and also suggest the existence of a wide range of combinations of above- and below-ground trait drought-responses among congeneric taxa.

**POS-WED-069**

**SHORT-TERM VERSUS PROLONGED SEVERE WATER STRESS AMONG SIX PRUNUS TAXA**

Brassica napus (canola) is the second largest oilseed crop grown worldwide with the annual production in Australia being around three million metric tons. The canola industry is globally affected by abiotic stress conditions such as drought, high salinity, frost and biotic stresses imposed by fungi, bacteria and insects. There is a pressing need for crop trait improvement in canola to better withstand these stress conditions. In this regard, genetic engineering could be a useful tool to introduce novel genes that have the potential to alleviate such stresses. The Acyl-CoA-Binding Protein (ACBP) family is thought to be involved in intracellular acyl-CoA ester transport and has been implicated in stress mediation in many organisms. Frost tolerant rapid-cycling B. napus and canola cv. Westar were developed by introducing the Arabidopsis thaliana ACBP6 cDNA using Agrobacterium-mediated gene transformation. Transgenic Brassica lines at T0 progeny were tested for frost tolerance in cold-acclimated and non-acclimated conditions in an upright fan-forced freezer with an electronic temperature controller. Electrolyte leakage, which arises from damage to biological membranes during freezing, was measured and found to be lower in the transgenic lines than in the wild type in non-acclimated conditions. Seed survival was higher in the transgenic lines after short-term and prolonged freezing in non-acclimated conditions. These findings indicate that the overexpression of ACBP6 is potentially useful in making canola crops more tolerant to frost in the field situations. This remains to be tested.
POSTERS

POS-WED-069
CHARACTERISATION OF CHITIN SYNTHASES FROM FUSARIUM GRAMINEARUM

Brain L.  
La Trobe Institute of Molecular Science, Kingsbury Dr, Bundoora, VIC 3086.

Fusarium graminearum (Fgr) is a devastating, agricultural pathogen; responsible for significant losses to cereal crops worldwide due to grain quality and yield reduction, as well as contamination of grains by carcinogenic mycotoxins. Resistance to the fungicides that are currently used in agriculture is spreading, limiting their sustainable use and leading to the urgent need to discover new fungicides with different mechanisms of action. We consider that chitin synthases (CHS) are excellent targets for new antifungal drugs, because chitin is essential for the integrity of the fungal cell wall and thus for survival and virulence of fungal cells. Furthermore, this polysaccharide is not made by plant and mammalian cells which will make antifungals that target chitin specific for fungi. No CHS inhibitors have been developed for control of human or agricultural fungal pathogens. One reason for this is the paucity of biochemical information on these important enzymes which has limited development of new antifungal molecules against this target. This aim of this study is to identify and biochemically characterise the full complement of chitin synthases in Fgr using a yeast model system. A combination of bioinformatics, functional complementation and microscopy has been used to identify homologs of the Saccharomyces cerevisiae CHS genes in Fgr. A chitin synthase assay is being conducted to biochemically characterise the Fgr CHS genes for the first time. The poster will outline progress and future directions.

POS-WED-070
SELECTING STRESS TOLERANT MALOLACTIC BACTERIA FOR AUSTRALIAN WINEMAKING

Costello P.J.,1 Bartowsky E.J.,2 Chambers P.J.,3, 5 Jordans C.J.1 and Schmidt S.A.1.
1The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064. 2Lallemand Australia Pty Ltd, PO Box 210, Edwardstown, SA 5039. 3Retired.

Malolactic fermentation (MLF) is an important step in the winemaking process, occurring in most red wines and certain white and sparkling wines. Malolactic starter cultures (typically Oenococcus oeni) are commonly utilised to enable greater control over the induction of MLF. However, MLF can often remain challenging, particularly under stressful conditions, with concomitant potential for quality and economic downgrades. Since most commercial malolactic starter cultures utilise strains of European origin, identifying robust Australian O. oeni isolates offers a potential advantage towards gaining greater reliability for MLF induction in Australian wine. In this study, a range of genetically diverse natural isolates of O. oeni, sourced from Australian wine regions, were phenotypically profiled for MLF performance and tolerance to wine stress factors using a robotic system. Miniaturized wine fermentations, undertaken in 96-well microplates, were used to screen different combinations of bacteria strain/stress factors for tolerances to alcohol, low pH and low temperature in red and white test wines. Potential stress-tolerant strains were further tested for MLF performance in lab- and winery-scale trials.

POS-WED-071
INVESTIGATION OF THE ALTERNATIVE RESPIRATORY PATHWAY IN HORDEUM VULGARE (BARLEY) DURING ABIOTIC STRESS

Dametto L.M.,1 Sweetman C.1, Day D.A.1, 5, Shavrukov Y.1, 5, Jenkins C.L.D.1 and Soole K.L.1
1School of Biological Sciences, Flinders University, Bedford Park. 5School of Life and Environmental Sciences, University of Sydney.

In plant mitochondria there exists an alternative respiratory pathway (AP) in addition to the classical, cytochrome-mediated electron transport chain (cyt pathway). The AP comprises two types of enzyme families; type II NAD(P)H dehydrogenases (ND), and alternative oxidases (AOX). Similar to the cyt pathway the AP involves the transfer of electrons from reducing power compounds such as NADH and NADPH. It is hypothesised that the AP enzymes function in moderating the level of important signalling compounds, reactive oxygen species (ROS) in the mitochondria as well as allowing release of adenylate control on metabolism. Abiotic stresses such as salinity, cold and drought lead to an increase in ROS in plant cells. In many studies the AP (especially AOX) is upregulated in response to abiotic and biotic stress. Many transgenic studies using model plant organisms have shown that overexpression of AOX allows plants to grow better under stress conditions than controls. There has been minimal research done on the AP in the important crop plant, Hordeum vulgare (barley). From an analysis of available barley genome databases and ESTs, four AOX and six ND genes in barley have been identified. Analysis of transcript and protein levels of these genes during an oxidative stress-inducing, potassium cyanide (KCN) treatment has shown that HvAox1a , HvAox1d1,HvAox1d2 as well as HvNdh3 are inducible in both roots and shoots. Immunoblot analyses have shown that AOX proteins in shoot tissue were upregulated in response to KCN and drought.

POS-WED-072
GENOME-WIDE IDENTIFICATION AND CHARACTERIZATION OF LONG NON-CODING RNAS FROM ARABIDOPSIS THALIANA IDAP SILIQUES

Do T.,1 Qu Z.,1 Jones A.,2 David R.,1 Adelson D.1 and Searle I.1
1Department of Genetics and Evolution, School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, 5005, Australia. 2The Australian National University, Canberra ACT 0200, Australia.

Long non-coding RNAs (IncRNAs) have critical regulatory roles in transcriptional and posttranscriptional regulation of development in plants and animals. In plants, the IncRNA landscape and subsequent functional analysis remain poorly understood during seed development. We performed strand specific RNA Illumina sequencing (RNA-seq) from 1 day after pollination (DAP) siliques from wild-type Arabidopsis thaliana and obtained >250,000,000 2x100 pair-end reads. Next, we developed a custom IncRNA pipeline and identified 2,808 novel IncRNAs that were either intergenic, antisense to protein coding genes, sense or antisense in introns. These IncRNAs ranged in length from 200nt to more than 40kb and overall had lower expression levels than protein coding genes. To identify endosperm specific IncRNAs, we utilized the INTACT system driven by an endosperm specific promoter, MPC, to facilitate cell sorting prior to RNA extraction, sequencing and IncRNAs annotation. We identified 408 IncRNAs specifically expressed in the endosperm. We validated the expression of a number of IncRNAs by QPCR. Next we knocked-down the expression of IncRNA, 1246 by using an artificial miRNA and observed that these plants have smaller seeds than wild type. These results provide an important insight into the role of IncRNAs during endosperm development and lay the foundation for future research.
CATALytically INactive CAs PROTEINS AS TRANSCRIPTIONAL ROADBLOCKS TO MODULATE GENE EXPRESSION

Donnelly A.J., Hao N., Dodd I.B. and Shearin K.E.
University of Adelaide.

A small number of DNA-binding proteins are known to hinder the progression of an elongating RNA polymerase along DNA by acting as a physical roadblock. These roadblocking proteins make promising genome engineering tools, with applications such as loss-of-function genetic screening and construction of synthetic gene networks. Studies have shown that a catalytically inactive Cas9 enzyme (dCas9) can reduce the level of gene expression by acting as a transcriptional roadblock. The simple two-component Cas9 system, requiring only the Cas9 gene and an engineered sgRNA, along with the ability to target virtually any gene, makes dCas9 a promising tool for programmable roadblocking. The effects of a number of cellular conditions, such as dCas9 concentration, the promoter strength of the target gene and the orientation and affinity of dCas9 binding, on dCas9 roadblocking are not yet fully understood. Through in vivo testing using simple modular systems within in Es. coli cells, we showed how increasing the promoter strength of the target gene reduces the repressive effect of dCas9, that increasing concentration of dCas9 in the cell enables greater roadblocking and that the correct orientation of dCas9 binding on the DNA is vital for effective transcription repression by dCas9. These tests can also be applied to other Cas family proteins, such as deactivated Cpf1 (dCpf1). dCpf1 differs from dCas9 most importantly by the location of the PAM site required on the target DNA. This difference may invert the orientation-dependent nature of roadblocking observed with dCas9. With this data, we aim to develop mathematical models which will allow us to extract biochemical parameters describing roadblock kinetics to assist improved manipulation of gene expression through optimising roadblocking conditions.

BIOPROSPECTING THE REGIONAL DIVERSITY OF AUSTRALIAN WINE MICROBIOTA

Hartmann L.A.2,1, Borneman A.1,2 and Schmidt S.1
1The Australian Wine Research Institute (AWRI). 2The University of Adelaide.

The many thousands of varied species and strains of microbiota represent a vast enzymatic reservoir of both function and potential application. With the advent of next generation sequencing, this biological reservoir has become accessible in ways that have not been afforded previously. In order to bioprospect grape associated microbiota for enzymes of interest in winemaking, total DNA was collected from two potential sources of variation - an un inoculated chardonnay grape must and composting grape marc. These environmental samples were shotgun sequenced and their respective metagenomes assembled. Homology based analysis was used on the predicted proteome of each metagenomic sample to predict enzyme function. Once functionally assigned, phylogenomic analysis was performed on two enzyme groups of interest (glycosyl hydrolases and proteases), which highlighted the presence of a large breadth of enzyme sequence diversity even within functionally close enzymes. Key representative enzymes from each group are now being functionally investigated via heterologous expression.

EFFECT OF PROCESSING ON THE FUNCTIONAL PROPERTIES AND DIGESTION BEHAVIOUR OF APPLE POMACE AND THE INTERACTION WITH EGGC DURING DIGESTION

Liu G.1,2, Ying D.Y.1, May B.3, Sanguansri L.1, Bird T.3 and Augustin M.A.1
1CSIRO Agriculture and Food, 671 Sneydes Road, Werribee, VIC 3030, Australia. 2College of Food Science, South China Agricultural University, 483 Wushan Road, Guangzhou, 510642, China. 3CSIRO Health and Biosecurity, Gate 13 Kintore Avenue, Adelaide SA 5000, Australia.

There is more than 25,000 tonnes of apple pomace a by-product of the apple juice processing, generated in Australia every year. Only a fraction of this apple pomace has been utilized. Apple pomace is rich in fibre, protein, polyphenols, and other nutrients and has a big potential to be transformed into highly nutritious food ingredients. In this study, we investigated the effect of food processing (eg. heat treatment, drying and extrusion) on the functional properties of the apple pomace and the digestion behaviours. We also investigated the interaction between apple pomace and other polyphenols, such as Epigallocatechin-3-Gallate (EGCG), to explore the possibility of using apple pomace as a carrier for EGCG. It was found that the physiochemical and functional characteristics were changed after extrusion, especially the extractable phenols and antioxidant capacity in extruded apple pomace were significant higher than that before extrusion. Apple pomace could improve the stability of EGCG in simulated gastrointestinal in vitro, and therefore, apple pomace could be a potential carrier for EGCG.

THE ALTERNATIVE RESPIRATORY PATHWAY OF A. THALIANA MITOCHONDRIA

Mcdonald A.D.1, Sweetman C.1, Day D.A.1,1 Jenkins C.L.D.1 and Soole K.L.1
1School of Biological Sciences, Flinders University, Bedford Park, SA 5042. 2School of Life and Environmental Sciences, The University of Sydney, NSW 2006.

The Alternative Pathway of mitochondrial electron transport has been implicated in a number of abiotic stress responses including heat, cold, drought, high light, and salt. Little is known of the role of this pathway in different cell types or how AP expression responds in different organs within the plant at different stages of development. This pathway includes type 2 dehydrogenases which are responsible for the oxidation of NAD(P)H on either side of the inner mitochondrial membrane to reduce the ubiquinone pool. Alternative Oxidase plays a complementary role in oxidising the ubiquinone pool completing the non-proton pumping pathway for NAD(P)H consumption. We are evaluating the expression of the AP in the leaves of the model plant A. thaliana with respect to cell type and developmental stage in response to salt or high light + drought stress. This increased resolution of the localization of the AP during these responses will provide an insight into the role of the AP and its components. Methods have been adapted from the literature for isolating intact transcript and protein from specific cell types of A. thaliana leaves to this end. Initial experiments have also examined the expression of the AP along the developing pedunle, showing a greater expression of AOX towards the base. No variation in expression between the leaves of individual plants after salt stress was observed, despite varied impact on growth.
POS-WED-077
INDUCIBLE HYPER-EXPRESSION AS A TOOL FOR METABOLIC ENGINEERING
Mortimer C.L.
Centre for Tropical Crops & Biocommodities, QUT.

Plant biosynthetic pathways produce a wide array of metabolites with exceptional economic and socioeconomic value. However, commercial production of these compounds is often limited by low accumulation levels in plants and their structural complexity is often a barrier to efficient in vitro or chemical synthesis. Metabolic engineering to alter the expression of biosynthetic genes and enhance the accumulation of target compounds is one solution to this problem; however, this approach can result in pleiotropic effects and the channelling of essential metabolites away from pathways critical for plant development. We are developing an In Plant Activated (INPACT) viral vector hyper-expression platform as a general tool for metabolic engineering to enhance target compound accumulation. Inducible expression, conferred by the INPACT system, avoids the negative effects that can arise with constitutive metabolic engineering strategies by separating altered gene expression and product accumulation from normal plant development. Hyper-expression should enhance productivity relative to standard gene expression systems. This approach to metabolic engineering has not previously been explored. To test this approach we are targeting the monoterpenoid indole alkaloid biosynthetic pathway in Madagascar periwinkle (Catharanthus roseus). Further, we are developing complimentary INPACT platforms to enable independent transcriptional control of multiple genes in the same elite plant.

POS-WED-079
PROGRAMMABLE DNA LOOPING IN VIVO
Hao N., Priest D.G., Dodd I.B. and Shearwin K.E.
Department of Molecular and Cellular Biology, The University of Adelaide.

DNA looping is a ubiquitous and critical feature of gene regulation. Although DNA looping can now be efficiently detected, tools to readily manipulate DNA looping are rare. In order to develop a simplified system for studying looping, we have recapitulated in E. coli the long range DNA looping typical of multicellular organisms, using a set of well-defined DNA looping proteins. In addition, we have developed a set of CRISPR-Cas based DNA looping reagents for the creation of programmable DNA loops. Cleavage-defective Cas9 (dCas9) proteins of different specificity were linked by heterodimerization or by translational fusion to create bivalent complexes able to link two separated DNA regions. After model-directed optimization, the reagents were validated using a quantitative gene regulation-based DNA looping assay in E. coli cells. Consistent with the modelling, overall looping efficiency could be significantly improved by expressing additional guide RNAs to create multiple DNA loops. Such reagents should allow manipulation of DNA looping in a variety of cell types, aiding understanding of endogenous loops and enabling creation of new regulatory connections.

POS-WED-080
ENGINEERING OF MICROALGAE, CHLAMYDOMONAS REINHARDTII FOR EFFICIENT RECOMBINANT PROTEIN PRODUCTION
Walia N.K., Commault A. and Ralph P.J.
University of Technology, Sydney.

Microalgae are increasingly being considered for recombinant protein production for a number of reasons including low cultivation costs, and the presence of post-translational modification mechanisms. Although, the effects of nutrients and light on algal biomass composition are well documented, little is known about the effect of changes to these culture conditions on recombinant protein production in Chlamydomonas reinhardtii. In this study, four different culture conditions have been investigated to improve the yield of the human recombinant protein, interferon alfa 2A (IFN α2A), in the green microalga, Chlamydomonas reinhardtii. Interferon alfa 2A is a small, soluble protein of 19.2kDa with anti-cancer and anti-viral properties and a global market value of approximately USD 2.5 billion. Three lines of C. reinhardtii producing IFN α2A were grown in phototropic (both 12h:12h and 18h:6h light:dark periods were tested), heterotrophic, mixotrophic conditions. The accumulation of IFN α2A was monitored by western blot over a 170 hours growth period in each condition. Preliminary data suggests that the production of IFN α2A is closely related to the consumption of acetate in mixotrophic conditions, as it peaks at mid-exponential phase and declines when the acetate in the medium is depleted when the algae entered the stationary phase. Though, this experiment is still in progress and we have only tested the mixotrophic condition till now, the results suggest that nutrients and light conditions do influence the production of recombinant IFN α2A in C. reinhardtii. The results of this study will help develop strategies for cost-effective production of high-value recombinant proteins in microalgae.
Salinity is a serious abiotic challenge facing Australian viticulture and winemaking, and is caused by high sodium (Na⁺) and chloride (Cl⁻) concentrations in soils and irrigation water. *Vitis vinifera* is moderately sensitive to salinity, and is especially sensitive to Cl⁻. Excessive Cl⁻ reduces vine water uptake, while hyper-accumulation of Cl⁻ in tissues causes plant growth inhibition, leaf burn and vine death. High Cl⁻ concentrations in grape berries leads to low quality wines with salty and soapy tastes, and wines that potentially exceed legal [Cl⁻] limits. These challenges can be minimised using *Vitis* spp. grapevine rootstocks with a high Cl-exclusion capacity, but further investigations are required to uncover the genetic mechanisms controlling this process. We identified genes that are differentially expressed between a good Cl⁻ excluding rootstock (140 Ruggeri) and a poor Cl⁻ excluder (KS1-40). Genes in the Nitrate/peptide transporter family VviNPF2.1 and VviNPF2.2, as well as genes in the Aluminium-activated malate transporter family VviALMT2 and VviALMT8, were more highly expressed in the Cl⁻ excluder 140 Ruggeri. Expression of VviNPF2.1-YFP and VviNPF2.2-YFP in Arabidopsis thaliana mesophyll protoplast revealed that both proteins are plasma membrane localised. *Xenopus laevis* oocytes expressing VviALMT2 and VviALMT8 suggested that both proteins are most permeable to NO₃⁻, while Cl⁻ is a substrate. Further experiments will aim to elucidate whether these candidate genes play a critical role in grapevine rootstock Cl⁻ exclusion, and may help to develop genetic markers for rootstock breeding.
WE ARE WHAT WE EAT: IDENTIFYING A REGULATORY CROSSTALK BETWEEN CENTRAL CARBON METABOLISM AND CELL DIVISION IN BACTERIA

Bottomley A.1, Mann R.1, Sonenshein A.2, Monahan L.1 and Harry E.1
1The three institute, University of Technology Sydney, Sydney, Australia NSW 2007. 2Sackler School of Graduate Biomedical Sciences, Tufts University, Boston, USA.

Bacterial cell division is driven by a cytokinetic ring - the Z ring - composed of polymers of the tubulin-like protein FtsZ. Z ring formation is tightly regulated to ensure faithful division, and several mechanisms have been described that influence the positioning and timing of Z ring assembly. One important but poorly understood aspect of division regulation is the need to coordinate cell cycle events with cell growth and nutrient availability; thus we aimed to address how bacteria use metabolic signals to determine conditions appropriate for cell division. We discovered that a pyk deletion (encoding pyruvate kinase involved in the last step of glycolysis to produce pyruvate) rescues the Z ring assembly defect of a temperature sensitive ftsZ mutant. This rescue is due to pyruvate, implicating it as a key metabolite in coordinating cell growth and division by regulating midcell Z ring formation. Our results support a model in which pyruvate levels are coupled to Z ring assembly via the enzyme that metabolizes pyruvate, the E2o subunit of pyruvate dehydrogenase, which localizes over the nucleoid in a pyruvate-dependent manner and may stimulate more efficient Z ring formation in response to nutrient availability. This is the first time that cell division is intimately linked to central carbon metabolism in the bacterium B. subtilis, and aids our understanding of how bacterial cells use nutritional signals to determine if they should ‘go forth and multiply’.

ROLE OF HOPX IN BMSC PROLIFERATION AND DIFFERENTIATION

Hng C.H.1,2, Camp E.1,2, Anderson P.1 and Grontelho S.1,2
1Mesenchymal Stem Cell Laboratory, Adelaide Medical School, University of Adelaide, SA. 2South Australian Health and Medical Research Institute, Adelaide, SA. 3Adelaide Craniofacial Unit, Women and Children Hospital, North Adelaide, SA.

Bone marrow-derived mesenchymal stem cells (BMSC) are self-renewing, multipotent cells that can give rise to multiple lineages including osteoblasts (bone), chondrocytes (cartilage) and adipocytes (fat). Interestingly, various pathways that promote BMSC osteo/chondrogenesis simultaneously suppress adipogenesis and vice versa. The BH3 transcription factor, TWIST-1 is highly expressed by BMSC and plays an important role in BMSC self-renewal and differentiation. Enforced expression of TWIST-1 enhances proliferation potential and lifespan of BMSC. It also enhances the adipogenic potential of BMSC yet inhibits chondrogenesis and osteogenesis. However, many of the underlying mechanisms mediating TWIST-1 regulation of BMSC growth and differentiation still remain poorly understood. In order to identify novel TWIST-1 gene targets involved in BMSC proliferation and osteogenic differentiation, microarray analysis was performed to compare the gene expression profile of BMSC which express either endogenous or enforced expression of TWIST-1 during growth culture conditions or undergoing osteogenic differentiation. One novel differentially expressed gene was HOPX. HOPX encodes for a hop homebox protein important in cardiogenesis. Currently, no known function of HOPX has been identified during BMSC growth or differentiation. We aim to determine whether HOPX is a novel target of TWIST-1 in BMSC and thus possibly be involved in mediating the effects of TWIST-1 on cell proliferation and lineage commitment.

NMD IN NEUROGENESIS, NEURONAL CELL ACTIVITY AND SYNAPTIC PLASTICITY

Johnson B.V.1,2, Sun Y.1,2, Homan C.1,2, Geucz J.1,2 and Jolly L.A.1,2
1Adelaide Medical School, Faculty of Health and Medical Sciences, University of Adelaide. 2Robinson Research Institute, University of Adelaide.

The Nonsense-mediated mRNA decay (NMD) pathway is a prevalent mechanism of post-transcriptional gene regulation. Patients lacking UPF3B, a core NMD factor, show intellectual disability implicating NMD in neurodevelopment and neuron function. We investigated the role of NMD in neurogenesis, neuronal maturation and mature neuronal cell activity. Both targeting Upf3b in mouse neurons with shRNA and investigating neural progenitors derived from a null animal revealed a promotion of progenitor cell self-renewal and delay of neural differentiation. Analysis of post-mitotic neurons lacking Upf3b revealed reduced morphological complexity and network activity (spike and burst rate), as assessed by multi-electrode arrays. Neuronal cell activity facilitates gene expression changes ultimately impacting neuronal plasticity. We treated control cultured cortical neurons with 50mM KCl, inducing depolarization to model neuronal activation, and observed the mRNA and protein of core NMD factors was reduced. Consistent with reduced NMD, NMD targeted mRNAs Gadd45b, Atf4, Snord, Mdga, Nrcam, Robo1 and Phgdh were elevated in response to neuronal activation. Retinoic acid (RA) inducing epigenetic changes are proposed to influence synaptic plasticity. We investigated candidate NMD targets in neurons which were known epigenetic regulators. Neurons depleted of the core NMD factor Upf1 with consequent NMD inhibition showed associated elevated expression of epigenetic regulators including Kdm5a, Kdm5c, Hdac1 and Gadd45b. These data support a requirement for NMD in brain developmental processes (neuronal progenitor differentiation) and mature neuronal cell function (activation and network activity). The observation NMD targets epigenetic modifiers supports an intriguing possibility that NMD links neuronal activity into epigenetic modification that may underpin lasting changes to synaptic plasticity, learning and memory.

USP9X MUTATIONS CAUSE A SPECTRUM OF NEURODEVELOPMENTAL DISORDERS UNDEPPINNED BY DISRUPTED SIGNALLING NETWORKS CRITICAL FOR BRAIN DEVELOPMENT

Jolly L.A.1, Johnson B.V.1, Kumar R.1, Burne T.1, Piper M.1, Wood S.A.1,4 and Geucz J.1
1University of Adelaide and Robinson Research Institute, Adelaide, Australia. 2Queensland Brain Institute, University of Queensland, Brisbane, Australia. 3School of Biomedicai Sciences, University of Queensland, Brisbane, Australia. 4Griffith Institute for Drug Discovery, Griffith University, Brisbane, Australia.

Loss of function mutations in the X-linked gene USP9X is a known in intellectual disability (ID) in females. In males, however only three missense mutations had been reported, and as such the involvement of USP9X in male ID remained less certain. We report 26 additional USP9X missense variants associated with male ID, with 21 mutations considered strong candidates for pathogenicity based on segregation and in-silico metrics. We describe an evolving phenotypic spectrum associated with USP9X missense mutations in males. In addition to ID and developmental delay, we found severe microcephaly, hypotonia, seizures, autistic behaviour, aggressiveness and visual impairment were frequently identified (64-100% of cases). Brain structural imaging showed evidence of disrupted white matter, thin corpus callosum and cortical malformations. Our USP9X knockout mouse model displayed overlapping brain structural features, and we now resolve severe learning and memory deficits highlighting its utility to understanding mechanisms of pathology. USP9X is a deubiquitylating enzyme capable of protecting substrates from proteasomal degradation. In embryonic brains of USP9X knockout mice, we show altered levels of multiple key substrates belonging to signalling pathways, and as such defective mTOR, WNT, NOTCH and TGFβ signalling is observed. Furthermore, we found these key substrates are disrupted in patient-derived fibroblast cells lines, suggesting defective signalling underlies pathology. In addition, proteomic analysis of the patient cell lines identifies USP9X as a regulator of the cytoskeleton. Collectively, our data demonstrate the involvement of USP9X in male ID and other neurodevelopmental disorders, and identify plausible mechanisms of pathogenesis.
POSTERS

POS-WED-089
INVESTIGATION OF A SECOND-HIT MECHANISM FOR GATOR1-RELATED FOCAL EPILEPSY
University of Adelaide, Department of Molecular and Cellular Biology.

Mutations in DEPDC5, NRPL2 and NRPL3, which make up the GATOR1 complex, have been found in a cohort of families with focal epilepsy. However the mechanisms of how these cause the disease remains elusive. In vitro studies have shown that GATOR1 functions to inhibit mTOR activation and hyperactivity of this pathway has independently been linked with epilepsy. mTOR dysregulation is therefore hypothesised to be the major factor in the pathology of GATOR1-related epilepsy. We have developed a functional assay using CRISPR null cell lines where the endogenous phenotype of hyperactive mTOR can be rescued by expressing the wildtype protein. GATOR1 mutations found in patients can therefore be screened for loss-of-function in the context of mTOR. Multiple germline mutations have been confirmed to have lost this function partially or completely. Somatic GATOR1 mutations have also been identified in patients and can be screened to investigate a ‘second-hit’ mechanism of disease, where seizures are proposed to result from a second somatic mutation in the brains of germline heterozygotes. To further investigate this hypothesis, we established a conditional mouse model for mutations in DEPDC5. Using CRISPR, we generated a floxed allele which following the unilateral electroporation of Cre into developing brains, recombines to result in discrete regions of null tissue. Preliminary results show increased mTOR signalling and increased soma size in regions where Cre-mediated deletion has been abolished. Together, these findings suggest that investigations using cell lines and mutant mice support the involvement of mTOR dysregulation in GATOR1-related epilepsy and a second-hit mechanism of disease.

POS-WED-091
PHENOTYPIC INDUCED FETAL HYPERGLYCAEMIA AND CANDIDATE GENE EXPRESSION
Oakes D.J., Howe A. and Ritchie H.E.
School of Medical Sciences, University of Sydney.

Objectives: Many women need to take phenytoin to control epilepsy and must remain on the drug during pregnancy. Yet it is the most common drug-induced cause of cleft lip and maxillary hypoplasia. The cause of the malformations is unknown but both hypoxia and hyperglycaemia are possible candidates. Using an animal model developed by the authors, we explored the effect of phenytoin on the expression of embryonic genes likely be involved in responses to these interlinked stresses. Method: Pregnant Rats were given a teratogenic dose of phenytoin during the critical period of craniofacial development (GD11) and embryos collected 2, 8 or 24 hours later. Embryos were collected from 4 animals at each time point. Embryos from each litter were dissected free of surrounding tissues, pooled, placed in RNAlater solution and stored at -20°C prior to RNA extraction. Real time quantitative PCR was performed using RNA extracts to determine the expression of the following genes: hypoxia pathway (HIF1a, VEGF), antioxidant pathways (SOD1, glucose transporter (GLUT1) and cell death (Tnfα). Results: Phenytoin-treated rats showed a spike in blood glucose 2-8 hours after dosing. This coincided with an increased expression of the glucose transporter gene and VEGF (1.4 and 1.4 fold increase respectively) in phenytoin-exposed embryos compared to controls. At 8 hours, HIF1α and SOD1 transiently decreased in the treated embryos. Surprisingly, there was no significant difference in expression patterns of Tnfα which might have been anticipated. Conclusions: Phenytoin is associated with increased maternal blood glucose for a prolonged period of time. In the genes selected for assessment, small increases in expression of the glucose transport gene as well as VEGF were observed. Surprisingly, genes associated with hypoxia and antioxidant pathways (HIF1 and SOD1) were downregulated compared to controls. This has been suggested that hyperglycaemia leads to localized increased oxygen consumption and resultant tissue hypoxia. Under normal circumstances, hypoxia promotes cell survival strategies which are initiated by increased expression of HIF1α but this system is not adequately activated in diabetic models. Our results support the hypothesis that hyperglycaemia is associated with a downregulation of HIF1α. Thus the mechanism of action of phenytoin in causing malformations may be a result of localised tissue hypoxia explaining the ameliorative effect of concomitant hypoxia and/or insulin treatment. Acknowledgements: The authors are grateful to the Australian Dental Research Foundation for their support.

POS-WED-092
THE ROLE OF COXSAKIE VIRUS AND ADENOVIRUS RECEPTOR (CXADR) IN MOUSE PLACENTAL DEVELOPMENT
Outhwaite J.E. and Simmons D.G.
School of Biomedical Sciences, The University of Queensland, St Lucia QLD 4072.

During mouse development the fetal and maternal circulations of the placenta increase their surface areas through branching morphogenesis and become intimately intertwined within the labyrinth layer to facilitate efficient nutrient exchange. Separating the two circulations and mediating exchange is a highly ordered multicellular barrier known as the interhaemal membrane (IHM). Interestingly, truly pluripotent trophoblast stem cells are lost just prior to formation of the labyrinth layer, and therefore, a more restricted labyrinthin progenitor population is thought to drive elaboration of the placental circulations and formation of the IHM later in gestation. However, the maintenance of these progenitors and how they contribute to the expansion of the labyrinth remains largely unexplored. Here we show that Cxadr, a cell adhesion molecule and viral receptor, is expressed in small clusters of proliferating trophoblast stem cells, overlapping with expression of labyrinth progenitor markers Rhox4b and Epcam. To explore the role of CXADR in placental development we deleted Cxadr in several contexts. Cxadr deletion in trophoblast stem (TS) cells in vivo altered the proliferation rate and morphology of TS cells. In addition, deletion of Cxadr in vivo is embryonic lethal between E11.5 and E12.5, with an adverse effect on the morphogenesis of the labyrinth layer detected as early as E10.5. These defects comprise significant placental growth restriction with decreased elaboration of the placental vasculature, impaired maintenance of labyrinth cell types and reduced IHM transport capability. However, as CXADR is also expressed within the developing embryo, a placenta-specific Cxadr deletion will be required to ascertain whether the placental defects in Cxadr mutant embryos contributes or accounts for their embryonic lethality.

POS-TUH-090
IDENTIFICATION OF REGULATORY GENES CONTROLLING SECONDARY CELL WALL BIOSYNTHESIS IN THE STEM OF SETARIA VIRIDIS
Nguyen T.T.M.1,2, Eamens A.L., Offer C.E.1 and Grof C.P.L.1
Centre for Plant Science, The University of Newcastle, Callaghan, NSW 2308, Australia. 1Department of Agriculture, Forestry and Fisheries, Vinh University, Nghean, Vietnam.

Lignocellulosic biomass of C4 bioenergy crops is being increasingly exploited as an attractive alternate source of renewable energy. The secondary cell wall (SCW) makes up the bulk of plant biomass and may be subjected to acid or enzymatic hydrolysis to release sugars for biofuel production. However, the complex structure of SCWs poses a significant challenge to cost effective enzymatic SCW digestibility. An elongating internode from Setaria viridis (green foxtail), a C4 bioenergy crop, was identified to use regulatory genes central to controlling SCW biosynthesis. The fifth internode, selected for a RNA-Seq investigation, exhibits four distinguishable developmental zones; a meristematic zone; a cell expansion zone, where primary wall development occurs; a transitional zone where cell expansion ceases and SCW deposition commences, and a maturation zone, where SCW deposition is ongoing. Bioinformatic analysis identified candidate transcription factors (TFs), belonging to MYB and NAC domain gene families, that are likely to regulate the expression of genes involved in SCW biosynthesis. In the transitional zone, the expression of 66 MYB and 16 NAC genes was substantially up-regulated (>1 log2 fold) as compared with the cell expansion zone. In particular, MYB42, MYB95-like, NAC53 and NAC63 were expressed most highly in the transition zone. This analysis further identified MYB42 as an outstanding candidate for further investigation as MYB42 expression increased 6-fold between the cell expansion and transitional zones. The S. viridis MYB42 is highly homologous to MYB42 of other C4 species, including maize, sorghum and switchgrass to indicate that the S. viridis MYB42 findings generated here will be readily transferable to other C4 species.
**POSTERS**

**POS-WED-093**

**THE A/HEJ MOUSE: DYSFUNCTION IN SEX DEVELOPMENT**

Robinson J.1,2, Graham A.1 and Kalitsis P.1,2

1Murdoch Children's Research Institute. 2The University of Melbourne.

The development pathway of the male gonad, the testis, occurs in response to the expression of the Y chromosome-linked Srygene. In mice, the expression of Sry is spatially and temporally regulated, with testsis development only inducible within a brief time window during embryonic development. Disruption or delay of Sry expression can result in XY sex reversal, or an intermediate gonadal phenotype, producing an ovotestis. The A/HeJ mouse strain was first reported in 2008 by Hunt et al, 'The mouse A/HeJ Y chromosome: Another good Y gone bad', and exhibits a disturbance in the male sex development pathway, with 4% of non-productive males presenting as overt hermaphrodites, and a further 17% having small testes with no epididymal sperm. It was hypothesised that there is a deletion at or near the Y chromosome centromere of A/HeJ and other closely related strains. Epigenetic and gene expression analyses have been performed on Sry to determine the cause of this sex development disturbance.

**POS-WED-094**

**THE REDUNDANCY AND FUNCTION OF SNAIL AND ESCARGOT WITHIN THE ADULT DROSOPHILA MIDGUT**

Savva E.1, Casagrande F.1, Siddall N.1, Abud H.2 and Hime G.1

1University of Melbourne, Department of Anatomy and Neuroscience, Parkville VIC 3010, Australia. 2Monash University, Department of Anatomy and Developmental Biology, Clayton VIC 3800, Australia.

Stem cells (SCs) are found in all regenerative tissues of the body, and are essential for normal tissue replacement during homeostasis and damage. They do so through their ability to divide indefinitely, whilst providing a new source of differentiated cells. Many cancers originate from a dysregulated SC, where too frequent proliferation forms the basis of a tumour. Therefore, refined regulation of SCs is essential for preventing disease. The Snail gene family of transcription factors has long been associated with SC maintenance in multiple tissues. Many different species from humans to Caenorhabditis elegans (roundworm), share structurally similar orthologues of Snail genes. We therefore chose Drosophila melanogaster as an animal model, and explored the role of the Snail family in the adult midgut, the fly intestine. Escargot mutations resulted in lost SCs. Knockdown of snail and escargot simultaneously had a more severe phenotype, with a more rapid, severe loss of SCs. Surprisingly, loss of snail intensity appeared to increase the number of proliferative cells. We also overexpressed snail in differentiated cells, and observed an increase in the number of mitosing SCs. This indicates a role for snail in differentiated cell signalling to SCs, to regulate proliferation. These results propose redundancy in function within the Snail family, highlighting how accumulated mutations produce a more severe phenotype. Previous studies have shown overactive escargot produces a tumourigenic phenotype, consistent with our loss-of-function snail mutations. Embryonic studies have shown cross-regulation between the genes does occur, and we show pilot evidence for this in the intestinal SCs. Molecular evidence in conjunction with known binding motifs and published literature, supports our hypothesis that snail represses escargot in SCs, to prevent dysregulation and SC overproliferation.

**POS-WED-095**

**DEFINING THE ROLE OF THE ATYPICAL CADHERIN FAT4 IN LYMPHATIC VASCULAR DEVELOPMENT**

Sutton D.1, Betterman K.1, Secker G.1, Kazenwadel J.1, McNeill H.2 and Harvey N.1

1Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, Australia. 2Lunenfeld-Tanenbaum Research Institute, Mount Sinai Hospital, Toronto, Canada.

Lymphatic vessels are vital for tissue fluid homeostasis, the absorption of dietary fats and immune cell trafficking. Our work aims to identify and define the mechanisms controlling lymphatic vascular growth and development during embryogenesis and in pathological settings. Here we describe a key role for the large atypical cadherin, FAT4, in the lymphatic vasculature. We identified FAT4 as a direct target gene of the transcription factor GATA2, established by our group to be of critical importance during lymphatic vascular development and mutations in which cause the human lymphoedema syndrome known as Emberger syndrome. Recently, FAT4 mutations were shown to underlie Hennekam syndrome, a human lymphoedema syndrome also caused by mutations in the Snail family, highlighting how accumulated mutations produce a more severe phenotype. Previous studies have shown overactive escargot produces a tumourigenic phenotype, consistent with our loss-of-function snail mutations. Embryonic studies have shown cross-regulation between the genes does occur, and we show pilot evidence for this in the intestinal SCs. Molecular evidence in conjunction with known binding motifs and published literature, supports our hypothesis that snail represses escargot in SCs, to prevent dysregulation and SC overproliferation.

**POS-WED-096**

**ELUCIDATING THE ROLE OF SOX9 TARGET GENES IN EMBRYONIC TESTIS DEVELOPMENT**

Symon A.1,2, Lavery R.1, Rahmoun M.1, Alankarage D.1, Bagheri-Fam S.1,2, Poullat F.1 and Harley V.1,2

1Hudson Institute of Medical Research, Melbourne. 2Department of Anatomy and Developmental Biology, Monash University, Melbourne. 3Institut de Genetique Humaine, Montpellier, France.

SOX9 is a key transcription factor and Sertoli cell fate determinant responsible for the differentiation of the gonad into a testis during embryonic development. Human SOX9 mutations cause Disorders of Sex Development (DSD) in XX males (SOX9 duplications) and XY females (SOX9 mutations/deletions), however most patients do not receive a definitive genetic diagnosis. We hypothesise that SOX9 target genes are candidate DSD genes. To identify SOX9 target genes, we undertook RNAseq analysis on mouse Sox9 knock-out gonads from embryonic day E13.5, when Sox9 is ablated in an intact Sertoli cell environment. We also performed Sox9 ChIPseq on wildtype E13.5 mouse testes and E90 bovine testes. 240 genes were downregulated in the Sox9 knockout testes, thus activated by Sox9. 4293 Sox9 ChIPseq peaks were common to the mouse and bovine testes. Overlapping the RNAseq and conserved ChIPseq datasets identified 119 genes whose gonadal chromatin is bound by Sox9, and whose gene expression is upregulated by Sox9. We are now elucidating the role of these genes in testis development of the testis using two main approaches. The first is to analyse the embryonic gonads of knockout mice available to determine whether Sox9 mediates testis development via the candidate gene. The second approach is to antagonise proteins with available drugs in an ex-vivo hanging-drop culture model where normally, E11.5 XY gonads will develop into testes with cords encompassing Sertoli cells after 3 days. These approaches are revealing unsuspected roles for Sox9 target genes in testis development. DSD patients with variants in these genes are being sought.
POS-WED-097

THE ROLE OF NEDD4 IN GONAD DEVELOPMENT

Windley S.P.1, Rastetter R.H.2, Neirinck Y.3, Nel S.3, Schwarz Q.4, Kumar S.1 and Wilhelm D.1

1Department of Anatomy & Neuroscience, The University of Melbourne, Parkville, VIC 3010, Australia. 2Department of Anatomy & Developmental Biology, Monash University, Clayton, VIC 3800, Australia. 3Department of Genetics, Medicine & Development, University of Geneva, Geneva CH-3211, Switzerland. 4Centre for Cancer Biology, SA Pathology, Adelaide, SA5000, Australia.

The neural precursor cell expressed developmentally down-regulated protein 4 (NEDD4) is an E3 ubiquitin ligase implicated in early fetal growth as well as the development of the nervous, cardiovascular and immune systems. Expression analysis has revealed high levels of Nedd4 in the embryonic testis and ovary. Constitutive ablation of Nedd4 has revealed drastic phenotypes in both sexes, with XY mice experiencing complete male-to-female sex reversal and XX mice exhibiting a significant delay in germ cell development. We are now utilising conditional ablation approaches to further elucidate the role of Nedd4 in both the germ cells and somatic cells of the fetal gonad. This is the first study to implicate Nedd4 in gonadal differentiation and is among the first studies to identify a role for post-translational modifications throughout gonadogenesis, opening up an exciting new area of research within the field.

POS-WED-099

PRE-TREATMENT OF ERYTHROCYTES WITH GARLIC AND TEA TREE OILS PROMOTES OXIDATION OF THE TYPICAL 2-CYS PEROXIREDOXIN 2 AND MAKES THE CELLS LESS SUSCEPTIBLE TO INFECTION BY PLASMODIUM FALCIPARUM

Al-Asadi S. and Schuller K.A.
School of Biological Sciences, College of Science and Engineering, Flinders University, Adelaide, Australia.

Plasmodium falciparum, the casual organism of the most deadly form of human malaria, lacks catalase and glutathione peroxidase enzymes and thus is highly dependent on peroxiredoxin (Prx) enzymes for $H_2O_2$ detoxification. In addition to its five Prx enzymes, human typical 2-Cys Prx2 protein is imported from the host erythrocyte into the cytosol of P. falciparum and half the $H_2O_2$ detoxification in P. falciparum is derived from the Prx2 protein. Here we have investigated the effects of pre-treatment of uninfected erythrocytes with increasing concentrations of garlic and tea tree oils on the redox/oligomerization state of Prx2 in the erythrocytes and on the P. falciparum parasitemia in the erythrocytes. Both oils were shown to be able to disrupt Prx2 redox state in pre-treated uninfected erythrocytes by promoting oxidised dimer formation. Garlic oil was a more potent promoter of the oxidation of the Prx2 protein than tea tree oil. The results also showed that pre-treatment of uninfected erythrocytes with the test oils significantly made erythrocytes less susceptible to infection by P. falciparum at 2nd generation ring stages (new infections). Garlic oil was more effective than tea tree oil in this respect. Additionally, both oils promoted irreversible oxidation of the Prx2 protein at 2nd generation ring stages and also appeared to promote oxidation of another sulphydryl group protein. Thus, oxidation of the Prx2 protein might partially be involved in decreasing the susceptibility of pre-treated uninfected erythrocytes to infection by P. falciparum. Results suggest that garlic and tea tree oils could be used as antimalarial treatments.

POS-WED-100

HOW DOES THE QACA EFFLUX PROTEIN MEDIATE MULTIDRUG RESISTANCE IN STAPHYLOCOCCI?

Chitsaz M.1, Stroeher U.1, Parker A.1, Bibi E.2, Sapula S.1 and Brown M.H.1
1Science and Engineering, Flinders University, Australia. 2Department of Biological Chemistry, Weizmann Institute of Science, Israel.

Multidrug efflux protein confers resistance to an exceptional number of antibiotics, many of which are commonly used as antiseptics and disinfectants. These structurally dissimilar compounds include monovalent cations, such as ethidium, benzalkonium and cetrimide and divalent cations, such as chlorhexidine, pentamidine and dequalinium. Importantly, the plasmid-borne qacA gene is carried by prevalent clinical isolates of Staphylococcus aureus. The 514 amino acids of the QacA exporter are arranged into 14 transmembrane segments (TMS) and it is classified as a member of the major facilitator superfamily (MFS) of transport proteins. Currently there is no high resolution structure of a 14-TMS MFS protein. Thus, a detailed molecular biochemical and biophysical approach has been undertaken to analyse the structure and function of QacA. Constructed QacA protein derivatives have been examined for a number of characteristics including; the ability to efflux substrates, solvent accessibility of target residues as gauged by fluorescein maleimide binding, as well as determination of the resistance profile to a representative set of six antimicrobial compounds. Deletion mutants of TMS of QacA identified regions critical for drug export and individual amino acids important for drug binding and translocation have been pinpointed using a site-directed mutagenesis strategy; to date over 2/3 of the residues have been mutated. A comprehensive picture of QacA-mediated drug efflux is now able to be formulated.

POS-WED-098

PROTEOGLYCANS AS POTENTIAL BIOMARKERS OF HUMAN STEM CELL NEURAL LINEAGE SPECIFICATION

Yu C., Okolicsanyi R.K., Oikari L.E., Griffiths L.R. and Haupt L.M.
Genomics Research Centre, Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia.

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) constituent proteoglycan (PG), is a major component. Recent findings by our group have identified specific heparan sulfate PG core proteins, syndecans and glypicans, as potential novel markers of neural lineage specification by demonstrating their role in hMSC and human neural stem cell (hNSC) maintenance and neural lineage commitment. hMSC populations (n = 3) were differentiated under neural lineage culture conditions through direct terminal differentiation and terminal differentiation via hMSC-induced neurosphere formation. RNA and protein were collected throughout differentiation at days 7, 14, 28, and 40 during neuronal and glial lineage differentiation conditions. Gene expression analysis by Q-PCR identified several significant gene expression changes in PGs between neural specific culture conditions and stages of differentiation. This data suggests PGs, in particular members of the syndecan (syndecans-1 and -4) and glypican (glypican-1 and -4) families, may be key players in hNSC neurogenesis. Further characterisation by transcriptome profiling and pathway analysis of hMSC neural cultures is in progress to identify the PG-mediated pathways regulating neural lineage specification. A deeper understanding of the complex and dynamic processes mediating human neurogenesis will provide important information to these central cellular process as well as enable advances in stem cell therapy for application to the understanding and repair of neurological disorders.
The rapid increase in the number of diabetic patients globally will increase the demand for recombinant insulin in the near future. This will challenge current manufacturing technologies with limited production capacity and high production costs. At present, insulin is being produced predominantly in *Escherichia coli* and Saccharomyces cerevisiae and is most commonly expressed as a soluble single chain propeptide, which then requires subsequent downstream processing including protein folding, proteolytic processing and purification. Current methods to produce single chain insulin that is correctly folded using *E. coli* are multistep and inefficient due to low refolding efficiency. The vortex fluidic device (VFD) is a continuous flow processing platform that accelerates synthetic transformations in dynamic thin films compared to conventional batch processing. The rapid rotation of a sample tube exposes reagents to high shear stress levels and Faraday waves delivering mechanical energy for mediating chemical and biochemical transformations, allowing processing beyond normal batch processing limits. It has applications in a number of diverse fields, including medicine, biochemistry, chemistry and materials science and allows the refolding of recombinant hen egg white lysozyme in minutes, more than 100 times faster than conventional overnight dialysis. Proteins that have been investigated thus far have been expressed in *E. coli*, followed by the folding step utilising the VFD. The use of the VFD has shown improved efficiency over conventional batch processing methods of oxidative folding, with refold times of minutes to hours versus days for other processing strategies. Investigation into the proteolytic processing and purification of protein products is also being undertaken. The utilisation of the VFD could significantly shorten times, lower costs, and reduce waste streams associated with protein expression and folding for this application to insulin production.

**POS-WED-102**

**INVESTIGATING THE PHYSIOLOGICAL ROLE OF K13 IN *PLASMODIUM FALCIPARUM***

Crisafulli E.M., Bridgford J.L., Tilley L. and Spillman N.J.

1Department of Biochemistry and Molecular Biology, Bio21 Molecular Science and Biotechnology Institute, The University of Melbourne, Melbourne, Victoria, Australia.

Artemisinin (ART) based combination therapy is the most effective antimalarial treatment for *Plasmodium falciparum* infections to date. However, ART resistance has begun to emerge across South-East Asia and is threatening to spread throughout Africa. K13 is a kelch-like protein in *Plasmodium*, and mutations in K13 are associated with ART resistance. However, the physiological function(s) of K13 in *P. falciparum* are unknown. The protein shares structural homology with human Keap1, a substrate adaptor for the ubiquitin proteasome system in animals, suggesting a similar role for K13 in *P. falciparum*. Substrates that will be ubiquitinated bind to the kelch domain of Keap1, and the BTB/POZ domain binds a complex of proteins, including a cullin-3 and E3 ligase, which acts to facilitate ubiquitination of the protein substrates. Protein ubiquitination is a signal for degradation by the proteasome. The notion that K13 performs a similar function to Keap1 is supported by evidence that upon ART exposure, K13 mutant parasites exhibit comparatively lower levels of polyubiquitinated proteins than wild type parasites. The aim of this project is to validate the role of K13 as a substrate adaptor in the ubiquitin proteasome system. We are identifying potential interacting proteins/substrates of K13 by creating knockouts of proteins in *P. falciparum* which share homology with components of the ubiquitin proteasome system, such as a cullin-like protein and a homologue of RXB1, an E3 ligase. Additionally, an APEX2 approach will be utilised to identify K13 proximal proteins. The preliminary results of these studies will be presented.

**POS-WED-103**

**EVOLUTION AND STRUCTURAL PLASTICITY OF EZRIN, A PROTEIN THAT COUPLES MEMBRANES TO THE ACTIN CYTOSKELETON**

Phang J.1, Harrop S.1, Duff A.2, Sokolova A.2, Goodchild S.1, Michie K.1, Bermeister A.1, Rathbone H.1 and Curmi P.1

1School of Physics, UNSW Sydney, NSW 2052, Australia. 2ANSTO, Lucas Heights, NSW 2234, Australia.

Ezrin is a member of the ERM (Ezrin-Radixin-Moesin) family of proteins that have been conserved through metazoan evolution. These proteins have dormant and active forms, where the latter links the actin cytoskeleton to membranes. ERM proteins have three domains: an N-terminal FERM (band Four-point-one ERM) domain comprising three subdomains (F1, F2 and F3); a helical domain; and a C-terminal actin-binding domain. In the dormant form, FERM and C-terminal domains form a stable complex. We have determined crystal structures of the FERM binding domain. In the dormant form, FERM and C-terminal domains form a stable complex. We have determined crystal structures of the dormant FERM:C-terminal domain complex of human ezrin. We observe a bistable array of phenylalanine residues in the core of subdomain F3 that is mobile in the active form and locked in the dormant form. As subdomain F3 is pivotal in binding non-cytoskeletal proteins and phospholipids, these transitions may facilitate activation and signalling. Full-length ezrin forms stable monomers and dimers. We used small-angle x-ray scattering to determine the solution structures of these species. As expected, the monomer shows a globular domain with a protruding helical coiled-coil. The dimer shows an elongated dumbbell structure that is twice as long as the monomer. By aligning ERM sequences spanning metazoan evolution, we show that the central helical region is conserved, preserving the heptad repeat. Using this, we have built a dimer model where each monomer forms one arm of an elongated anti-parallel coiled-coil with domain-swapped FERM:C-terminal domain complexes at each end. The model suggests that ERM dimers may bind to actin in a parallel fashion.

**POS-WED-104**

**STRUCTURE-ACTIVITY RELATIONSHIP OF INT131 ANALOGUES FOR PPARY-TARGETED ANTIDIABETICS**

Frick R.L.1, Kamenecka T.M.2, Griffin P.R.2 and Bruning J.B.1

1University of Adelaide. 2Scripps Research Institute, US.

Peroxisome Proliferator-Activated Receptor γ (PPARY) is a ligand-activated nuclear receptor which plays a key role in fatty acid and glucose homeostasis. PPARY is the molecular target for type 2 diabetes mellitus (T2DM) therapeutics known as the T2Ds (thiazolidinediones), drugs that offer robust clinical benefit in terms of normalising fasting glucose. However, the T2Ds, which are full agonists of the receptor, have been confounded with significant side effects. In recent years, it has been shown that partial agonists of PPARY have displayed similar insulin sensitising efficacy as the full agonist T2Ds, but lack many of the undesirable side effects of the full agonists. One such partial agonist, INT131, has shown potent insulin-sensitising actions with reduced side effects as compared to the T2Ds. To probe the structure-activity relationship (SAR) of the INT131 scaffold, 14 analogues of INT131 were synthesised. SAR studies of the analogues revealed compounds with higher transcriptional potency for PPARY as well as identification of moieties of the INT131 scaffold key to high transcriptional potency. The sulphonamide linker is absolutely critical to activity, substitutions at position 4 of the benzene ring A were associated with higher transcriptional activity, substitutions at position 2 of benzene ring A aided in tighter packing and activity, and the ring type and size of ring A was correlated to the degree of activity.
SIGNALLING AND EFFICACY AT CLASS B, G PROTEIN-COUPLED RECEPTORS

Furness S.G.B., Liang Y.-L., Nowell C.J., Wootten D. and Sexton P.M.
Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences and Department of Pharmacology, Monash University, Parkville, 3052, Victoria, Australia.

The observation, that different ligands acting at the same G protein-coupled receptor (GPCR) can illicit responses varying from reduced basal activity (inverse agonism) to maximal activation at low receptor occupancy is explained pharmacologically & mathematically through the use of the term efficacy. This is an extremely useful pragmatic approach. It has allowed pharmacological comparison of receptor ligands and as the basis for understanding agonist structure-activity relationships. Contrastingly, there is little data addressing the underlying molecular basis of efficacy. Differential efficacy is thought to principally occur via stabilization of distinct receptor conformations by individual ligands, which regulates effector protein recruitment. We use native PAGE, including in-gel FRET (Forster Resonance Energy Transfer) along with G protein BRET (Bioluminescence Resonance Energy Transfer) to demonstrate that efficacy at the calcitonin GPCR (CTR) is also mediated by ligand-dependent alterations to G protein conformation. Agonist promoted differences in effector conformation result in differential guanine nucleotide exchange and G protein activation. Apparent affinity and on-rate measurements for G protein association to the active receptor didn’t correlate with ligand efficacy. Chimeric agonists demonstrate that ligand residency time isn’t a driver for differential efficacy at the CTR. Instead, we observe ligand-dependent differences in guanosine-5-triphosphate affinity and on-rate at the tertiary complex. These are due to conformational differences in the receptor bound G protein hetero-trimer, resulting in differential receptor-residency times for the hetero-trimeric G protein, as measured using live-cell TIRF (Total Internal Reflection Fluorescence) microscopy. Downstream, we use live-cell CFP sensors to demonstrate differential CAPM accumulation rates in real time. These results are informed by our recent determination of the active ternary complex structure of CTR by single-particle cryo-electron microscopy. This study extends basic assumptions about agonist efficacy at the CTR, moving from a paradigm driven by receptor conformation, to one that considers the influence of the agonist-receptor complex on effector protein conformation.

CDNA SEQUENCING OF GHF9 ENDO-BETA-1,4-GLUCANASES FROM THE CHRISTMAS ISLAND LAND CRAB, GECARCOIDEA NATALIS

Gray M.1, Linton S.M.1 and Allardyce B.J.2
1School of Life and Environmental Sciences, Deakin University. Institute for Frontier Materials, Deakin University.

The Christmas Island red crab, Gecarcoidea natalis is mainly herbivorous consuming mostly brown leaf litter. On a brown leaf diet, this species is able to achieve respective digestibilities for cellulose and hemicellulose of 38-43% and 49-58%, using endogenously produced cellulase and hemicellulase enzymes. Three endo-β-1,4-glucanase (classic cellulase) isoforms, with molecular masses of 43, 47.4 and 53 kDa, have been purified and characterised from this species. The sequence encoding the endo-β-1,4-glucanase have yet to be elucidated and hence this formed the aim of the study. Three different transcripts, two complete and one partial were sequenced from cDNA prepared from RNA isolated from the midgut gland (tissue responsible for the production of digestive enzymes). An open reading frame of 1383 bp was translated. Translated this would produce a putative protein of 460 amino acids, with a 16 amino acid peptide sequence. The molecular mass of the mature protein is estimated to be 47,6-47,7 kDa and this closely matches that of the 47.7kDa isozyme. Hence this isoenzyme may be the product of the sequenced cDNA. The enzyme belonged to glycosyl hydrolase family 9 given it contained features which are characteristic of this family; these are catalytic and binding domains and a predicted tertiary structure of an a/β barrel fold and an active site which can accommodate five glucose residues. The presence of multiple endo-β-1,4-glucanase transcripts suggests gene duplication. These transcripts encode a suite of enzymes expressed and have varying basal activity. This in turn may increase the efficiency of cellulose digestion. The GHF9 endo-β-1,4-glucanase is also widely expressed within the Crustacea as its CDNA sequence was confirmed in other species. The potential evolution of the enzyme during the colonisation of land by decapod crustaceans (crabs) and the adoption of a terrestrial leaf litter diet will also be discussed.

STRUCTURAL ANALYSIS OF A PROTEASE ACTIVATOR IN THE MALARIA PARASITE PLASMODIUM FALCIPARUM

Gillett D.L.1, Wong W.2, Metcalfe R.1, Cowman A.F.3, Griffin M.D.1, Tilley L.M.1 and Xie S.C.1
1Department of Biochemistry and Molecular Biology, The University of Melbourne, Bio21 Institute, Parkville, Melbourne, Australia. 2Infection and Immunity Division, The Walter and Eliza Hall Institute, Parkville, Melbourne, Australia.

The proteasome represents one of the key protein degradation pathways in all organisms. Access to the proteasome’s internal chamber is restricted by default and activator complexes are responsible for greatly increasing substrate access and subsequent degradation. One class of activators are the 11S activators which operate in a ubiquitin and ATP independent manner and are formed by seven PA28 subunits. While some evidence suggests a role in antigen presentation in humans, the precise function of 11S activators is not understood. Plasmodium falciparum, the protozoan parasite responsible for the majority of fatal cases of the disease malaria, also possesses an 11S activator (PfPA28) which has not been previously characterised. The P. falciparum proteasome (P120S) is involved in the parasite’s response to the frontline antimalarial drug artemisinin, and understanding how the 11S activator modulates the activity of the 20S particle may further elucidate this mechanism. Here, we purify recombinant PfPA28 and show that it activates purified endogenous Pf20S using fluorogenic peptide substrates. We show that PfPA28 readily forms a single and double capped complex with Pf20S using native PAGE and electron microscopy (EM). We also present our current progress in attaining a high resolution structures of the PfPA28 heptamer using X-ray crystallography and of the PfPA28-Pf20S complex using Cryo-EM.

TOWARDS A FERMENT-ACTIVE PROTEASE FOR WHITE WINE PROTEIN STABILITY: A SUNFLOWER ASPARAGINYL ENDOPROTEASE AND A BOTRYTIS CINEREA PROTEASE

McRae J.M.1, Warnock N.I.2, Schmidt S.A.1, James A.M.3, Mylne J.S.3, Anderson P.1 and Smith P.A.1
1Australian Wine Research Institute, PO Box 197, Glen Osmond SA 5064, Australia. 2Finders University, School of Biological Sciences, Sturt Rd, Bedford Park, SA 5051, Australia. 3The University of Western Australia, School of Molecular Sciences & The ARC Centre of Excellence in Plant Energy Biology, 35 Stirling Highway, Crawley, Perth 6009, Australia.

White wine production requires the removal of thiamatin-like proteins (TLPs) and chitinas to prevent haze formation in wines post-bottling. This can be achieved via bentonite addition or with aspergillopespin I and II addition in conjunction with heat treatment (70°C, 1 min); however, ensuring wine clarity without additional processing steps remains a priority. Two proteases, sunflower (Helianthus annuus) asparaginyl endopeptidase, HaAEP1, and Botrytis cinerea protease, BcAP8, were assessed for efficacy in reducing the concentrations of TLPs and chitinases in grape juice prior to fermentation. Concentrations of these haze-forming proteins were measured using RP-HPLC against a standard curve of thiamatin. The haze-forming potential of wines were assessed by measuring the change in turbidity of the sample before and after heating at 80°C for 2 h. BcAP8 addition (12.5 mg/L) to grape juice reduced the concentration of haze-forming proteins and the formation of haze in Chardonnay wines by around 20% compared to the control wines. HaAEP1 addition (10-30 mg/L) decreased the concentration of haze-forming proteins in Semillon juice by around 50% after 7 days at 33°C compared to controls. The activity of HaAEP1 and BcAP8 in grape juice suggested that these proteases may be candidates for directed evolution with the goal of improving efficacy towards haze-forming proteins during fermentation.
INVESTIGATING THE FUNCTIONAL ROLE OF UNIQUE PROTEIN INSERTS IN PLASMODIUM FALCIPARUM ENZYMES

Petrinolis M., Menz I.R. and Herbert J. Flinders University of South Australia, Sturt Rd, Bedford Park SA 5042.

The sequencing of the malarial genome has revealed that many of the protein-coding genes, have regions which are not conserved in other organisms. These unique Plasmodium specific protein inserts are translated and occur in approximately 17.3 % of protein coding genes in the genome. Many hypotheses have been put forward for the possible function of these inserts but there is a lack of experimental evidence to support these. If these inserts do have a function, we hypothesise that the function would be mediated by an interaction between insert region of the protein and a component of the malarial cell. To investigate this we selected a representative set of proteins containing a single unique protein insert. These proteins were expressed in E. coli along with variants where the inserts had been replaced but the protein had been demethylated to be functional. Isothermal calorimetry and Biacore were used with these recombinant proteins to identify interaction between the unique inserts and components of Plasmodium cell extracts. For some of the selected proteins specific interactions between the insert and components of the cell were identified. Characterisation of these interacting cellular components will provide possible insight to the function of these unique protein inserts.

POSTERS

POS-WED-109

SHORT OPEN READING FRAMES AND THEIR CODING POTENTIAL - AN UPDATE

Phung T.1, Nourovs A.2, Menschaert G.2 and Rothnagel J.A.1

'1School of Chemistry & Molecular Biosciences, The University of Queensland, Brisbane, Australia. 2Laboratory of Bioinformatics & Computational Genomics, Ghent University, Ghent, Belgium.

More than ten years ago, we postulated that the upstream open reading frames (uORFs) found in a majority of mammalian mRNAs might be a novel source of small peptides with independent biological activity. We termed these uORF–encoded peptides uPEPs and we used simple bioinformatic approaches to identify about 1000 of these with sequence conservation, at the amino acid level, between human and other vertebrates. We tested several of these in cell biology assay and observed that most exhibited unique localization patterns. We then used mass spectrometry to find physical proof that uPEPs actually existed. To date, we only have proteomic evidence for a handful of the uPEPs. Intriguingly, we observed many hundreds of peptides arising from short ORFs (sORFs) on non-mRNAs including long non-coding and antisense RNAs. These sORFs, arbitrarily defined as smaller than 100 codons, were often missed (or down-right ignored) in genome annotations. Moreover, nearly 25% of the short peptides (sPEPs) identified in our study, originated from small ORFs (sORFs) such as CUG and UUG which brings an added complexity to the identification of sORFs using in silico methodologies. We then corroborated our mass spectroscopy studies by employing ribosome profiling: the translation wide sequencing ribosome protected RNA (Ribo-seq) that can identify translating ribosomes as well as those initiating at translation start sites. We used publicly available and in-house generated Ribo-seq data to identify cognate and alternative translation initiation sites with sub-codon to single-nucleotide resolution. Furthermore, we discovered several putative coding sORFs in different categories of ncRNAs. This proteogenomics approach has greatly enhanced the identification of bona fide translatable sORFs in eukaryotes.

POS-WED-110

CIRCULATING AUTOANTIBODIES AS BIOMARKERS OF EARLY STAGE, HIGH GRADE SEROUS OVARIAN CANCER

Wilson A.L.1, 2, Moffitt L.R.1, 2, Plebanski M.2 and Stephens A.N.1, 2

1Hudson Institute of Medical Research, VIC. 2Monash University, VIC.

High grade serous epithelial ovarian cancers (HGSOCs) are typically diagnosed at an advanced stage, and account for ~90% of all ovarian cancer related deaths. Early diagnosis, prior to extra-ovarian spread, is associated with improved survival. There remains an unmet clinical need to identify novel biomarkers for the early-stage diagnosis of HGSOCs. Anti-tumor immune responses generate auto-antibodies (AABs) well before the clinical manifestation of disease. We have performed a pilot study to identify AABs as potential biomarkers for the detection of early-stage HGSOC. Plasma samples from patients with malignant (early or late stage cancer), benign or no disease (n=20/group) were collected, and randomly assigned to “discovery” or “validation” cohorts. The presence and reactivity of AABs (IgG, IgA and IgM) in these groups were determined using high-content protein arrays. Functional enrichment analyses were performed using Ingenuity Pathways Analysis and Gene Ontology. Early-stage HGSOC can utilise a wide range of NTPs, with a marked preference for cytidine triphosphate (CTP). To further explore the penultimate step of biotin synthesis - the conversion of diaminopelargonic acid (DAPA) to dethiobiotin using a nucleotide triphosphate (NTP). Using surface plasmon resonance (SPR) and enzyme activity assays, we have demonstrated that DTBS can utilise a wide range of NTPs, with a marked preference for cytidine triphosphate (CTP). To further explore the unique DTBS active site, 57 cytidine analogues and 93,904 fragment structures were screened in silico against DTBS using AutoDock Vina, and 26 compounds with the highest predicted binding affinity were purchased. Using a high concentration crystal soaking approach, ligand bound crystal structures were obtained for 4 cytidine analogues and 1 fragment. Binding analogues differed minimally from cytidine, consolidating our hypothesis that it is the preferred nucleoside. However, the high concentrations required, in combination with SPR data, implicates the crucial role of triphosphates in NTP binding. The structure of the fragment soaked DTBS crystal revealed a degradation product (B9D) bound to the DAPA pocket. Serendipitously, B9D constitutes a convenient chemical scaffold for the development of more potent inhibitors of DTBS. We are collaborating with chemists to optimise B9D binding affinity by engineering interactions to replicate those of DAPA and NTP triphosphates, with one structurally guided chemical modification achieving a 10 fold increase in affinity. This collaboration is also currently exploring B9D–cytidine linkage strategies, in an attempt to attain super-additive affinity increases. Different linker chemistries are currently being investigated in order to replicate the crucial triphosphate interactions.

POS-WED-111

ACTIVE SITE PROBES REVEAL A STRATEGY FOR THE INHIBITION OF BIOTIN BIOSYNTHESIS IN MYCOBACTERIUM TUBERCULOSIS

Thompson A.P.1, Salaemae W.1, Gaiser B.2, Lee K.J.3, Abei A.2, Booker G.W.1, Brusing J.B.1, Polayk S.W.1 and Wegener K.L.1

1School of Biological Sciences, University of Adelaide, Adelaide, South Australia, 5005. 2School of Chemistry, University of Adelaide, Adelaide, South Australia, 5005.

Tuberculosis (TB) remains a major global health problem with one third of the world's population infected by the causative pathogen Mycobacterium tuberculosis (Mt). Biotin biosynthesis has been identified as a promising target for anti-TB therapeutics, as it is Mycobacterial species' only means of acquiring this essential cofactor. Dethiobiotin synthetase (DTBS) catalyses the penultimate step of biotin synthesis - the conversion of diaminopelargonic acid (DAPA) to dethiobiotin using a nucleotide triphosphate (NTP). Using surface plasmon resonance (SPR) and enzyme activity assays, we have demonstrated that DTBS utilises a wide range of NTPs, with a marked preference for cytidine triphosphate (CTP). To further explore the unique DTBS active site, 57 cytidine analogues and 93,904 fragment structures were screened in silico against DTBS using AutoDock Vina, and 26 compounds with the highest predicted binding affinity were purchased. Using a high concentration crystal soaking approach, ligand bound crystal structures were obtained for 4 cytidine analogues and 1 fragment. Binding analogues differed minimally from cytidine, consolidating our hypothesis that it is the preferred nucleoside. However, the high concentrations required, in combination with SPR data, implicates the crucial role of triphosphates in NTP binding. The structure of the fragment soaked DTBS crystal revealed a degradation product (B9D) bound to the DAPA pocket. Serendipitously, B9D constitutes a convenient chemical scaffold for the development of more potent inhibitors of DTBS. We are collaborating with chemists to optimise B9D binding affinity by engineering interactions to replicate those of DAPA and NTP triphosphates, with one structurally guided chemical modification achieving a 10 fold increase in affinity. This collaboration is also currently exploring B9D–cytidine linkage strategies, in an attempt to attain super-additive affinity increases. Different linker chemistries are currently being investigated in order to replicate the crucial triphosphate interactions.
POSTERS

POS-WED-113

TWO GROUPS OF NOVEL PROTEINS ENCODED BY GENES IN ARS OPERONS OF BACTERIA

Tran A.P., Sifice I. J. and Zhang R.
School of Biological Sciences, University of Wollongong, NSW, Australia.

Arsenic (As) is one of the most toxic metalloids that is harmful for all organisms. In response, living organisms have developed several strategies for As detoxification. In bacteria, arsenic resistance is usually mediated by the proteins encoded in arc operons. Several arc genes are already well characterised such as arcsR, B, A, C and D, however there are still many other genes in arcoperons that are not characterised. Here we report our investigations on two groups of such genes encoding putative dual specificity phosphatases (DSP) and glyceraldehyde-3-phosphate dehydrogenases (GAPDH), respectively. Our search has located dozens of putative DSP and GAPDH genes in arc operons. They appear widely spread in different groups of bacteria. The putative DSPs sharing the catalytic motifs with known DSPs are also homologous to eukaryote arsenate reductases (ACR2), these putative DSPs formed some distinct groups on phylogenetic tree. Interestingly, the putative GAPDHs encoded in the arc operons contain not only all the common motifs of GAPDH but also some distinct features compared with other bacterial GAPDH proteins. A phylogenetic analysis indicated that these arc-associated GAPDHs form a discrete group in the tree, suggesting that they may share the same ancient origin but have acquired a different function through evolution. The involvement of these two groups of proteins in arsenic resistance and their biochemical function are under investigation.

POS-WED-115

MICRORNAS ENHANCE ANTICANCER PROPERTIES OF BUTYRATE IN COLORECTAL CANCER

Ali S.R.1, Humphreys K.J.1, Simpson K.2, McKinnon R.A.1 and Michael M.Z.1
1Flinders Centre for Innovation in Cancer, Flinders University, Flinders Medical Centre, Adelaide, South Australia 5042. 2Victorian Centre for Functional Genomics, The Peter MacCallum Cancer Centre, Melbourne, Victoria.

The dysregulation of microRNAs in colorectal cancer contributes to tumour development and progression. Diet may be a contributing factor to colorectal cancer risk, and there is evidence to suggest that the fibre fermentation product, butyrate, has anticancer properties achieved through epigenetic changes in gene expression. Previous studies have demonstrated that butyrate can alter microRNA expression in colorectal cancer; however, the ability of microRNAs to enhance these anticancer properties requires further investigation. This project aimed to determine whether microRNAs can sensitise colorectal cancer cells to butyrate, with this mechanism thereby enhancing the anticancer effect. High throughput functional screens were used to systematically identify miRNAs with the ability to sensitise HCT116 colorectal cancer cells to butyrate by inducing anti-proliferative and pro-apoptotic effects. Validation of this effect was performed using real-time cell analysis systems. miR-125b and miR-1227 showed particularly significant (P value <0.05) exacerbation of the butyrate response. Pathway analysis highlighted potential miRNA target genes involved in cell growth, cell death, and cancer related pathways. RT-PCR and western blotting revealed reduction in transcript and protein levels respectively, of cancer-associated predicted target genes involved in key cell growth pathways including WNT signalling. MicroRNA binding sites in the 3'UTR of the predicted target genes were identified and are being validated using target protectors and luciferase assays. This study is the first unbiased screen to identify microRNAs that enhance the anticancer effects of butyrate in colorectal cancer cells.

POS-WED-114

REVERSING ANTIMICROBIAL RESISTANCE WITH EFFLUX PUMP INHIBITORS

Venter H.1, Mowla R.1, Ma S.1, Xiang L.2 and Semple S.J.1
1School of Pharmacy and Medical Sciences, University of South Australia, Adelaide 5000, Australia. 2School of Pharmaceutical Sciences, Shandong University, Jinan 250012, China.

Drug efflux pumps confer multidrug resistance on bacteria by transporting a wide spectrum of structurally diverse antibiotics. Efflux pumps are also necessary for the acquisition of resistance. Due to the substrate promiscuity of drug efflux pumps they are not only involved in antimicrobial resistance but also play a central role in virulence and biofilm formation. Yet, despite their crucial role in bacterial pathogenesis and multidrug resistance, there are currently no inhibitors of drug efflux pumps in clinical use. We have used the archetypal transporter AcrB from *Escherichia coli* as model efflux pump as AcrB is widely conserved throughout Gram-negative organisms. AcrB inhibitors were identified by in silico screening of drug libraries and activity-guided-fractionation of medicinal plants. Potential EPIs were tested for their antibacterial action, ability to potentiate the action of antibiotics and ability to inhibit substrate efflux by AcrB. Target-specific activity was confirmed by verification that the compounds do not act on efflux pump deleted strains and by testing off-target effects such as membrane permeabilisation. Cytotoxicity was determined using the RealTime-Glo cell viability assay and the kinetics of inhibition were probed with surface plasmone resonance. The first screen identified 2-naphthamides as putative EPIs. Second generation compounds were synthesised based on the most active compounds and their structure-activity relationship were determined. Additionally, activity-guided fractionation of Indigenous medical plants identified prenylated flavonoids that could synergise with antibiotics against an antimicrobial resistant bacterium. In this study we have designed and synthesised novel chemical compounds and identified new phytochemicals with great potential for further optimisation as inhibitors of drug efflux pumps to reverse antimicrobial resistance.

POS-WED-116

INVESTIGATING TRANSLOCATOR PROTEIN (TSP0) FUNCTION IN NEURODEGENERATIVE DISEASES

Asih P.R.1, Ke Y.D.2 and Ittner L.M.1
1Dementia Research Unit, Department of Anatomy, School of Medical Sciences, Faculty of Medicine, UNSW, Sydney, NSW, Australia. 2Motor Neuron Disease Unit, Department of Anatomy, School of Medical Sciences, Faculty of Medicine, UNSW, Sydney, NSW, Australia.

Background: Translocator protein (TSP0), an outer mitochondrial membrane is increased in microglial cells in disease states. TSP0 has been thought to be fundamental for cholesterol transport; a prerequisite for sterologenesis; apoptosis, inflammation, cell proliferation, and heme biosynthesis. At present, its ligands have served as an imaging marker of neuroinflammation and as an attractive drug target in neurodegenerative diseases ranging from anxiety to AD. However, recent knockout studies have challenged these roles. In light of the controversy surrounding the function of TSP0, we investigated TSP0 function in a knockout model. Methods: TSP0 knockout mice crossed with TAUS5/2 mice (P301S tau transgenic mice that resemble features of human FTD and AD) were bred to reach 2.5 months, 4.5 months and 8.5 months. At each age, both male and female mice underwent extensive behavioural tests battery such as elevated plus maze (EPM) and motor testing. Starting at 4.5 months, these mice underwent Morris water maze (MWM) and open field (OF) tests. Results: While TAUS5/2 mice presented with disinhibition-like behaviour in EPM and hyperactivity in OF, both adult and aged TAUS5/2 mice with partial and complete deletion of TSP0 presented with significantly less disinhibition, which was not due to anxiety. Conversely, TAUS5/2 mice with complete TSP0 deletion showed delayed learning performance. Conclusions: The disinhibition seen in TAUS5/2 mice is diminished in the absence of TSP0, indicating that TSP0 may mediate disinhibition in this model.
Malignant pleural mesothelioma is an aggressive and fatal malignancy originating in pleural mesothelial cells with median survivals of approximately 12 months following diagnosis. Recently, anti-angiogenic therapies have been trialed with only a modest effect. This may be, in part, due to alternative mechanisms of tumour vascularisation such as vasculogenic mimicry (VM), the ability of tumour cells to form fluid carrying vascular channels. Curcumin, a polyphenol extracted from the spice turmeric, has numerous anti-cancer and anti-inflammatory properties. Our aims were to investigate the effect of curcumin on vasculogenic mimicry and to determine if curcumin acts by disrupting microRNA profiles of mesothelioma cells. Mesothelioma cell lines and patient-derived primary mesothelioma were utilized for in vitro experimentation. A matrigel tube formation assay was performed to assess if curcumin could inhibit VM in vitro. Small non-coding RNA sequencing was performed to determine if curcumin (20μM) had an effect on microRNA expression. Curcumin inhibited the ability of mesothelioma cells to perform VM in vitro. The microRNA expression profiles differed greatly between each mesothelioma sub-type, however minimal curcumin-induced change was observed. Differential expression analysis revealed mir-486, a cancer-related microRNA that is down regulated in mesothelioma, was upregulated when treated with curcumin compared with the untreated control. We also observed down regulation of mir-4485 and mir-190b when treated with curcumin compared with the untreated control. Curcumin is able to inhibit vasculogenic mimicry in vitro, which may be, in part, due to altered microRNA profiles. We will perform qPCR to validate changes observed in the RNA sequencing.

TRM4B IS REQUIRED FOR 5-METHYLCYTOSINE ON mRNAs AND NON-CODING RNAs IN ARABIDOPSIS THALIANA

Li J.
The University of Adelaide.

RNA has co-evolved with numerous post-transcriptional modifications to sculpt interactions with proteins and other molecules. Methylation is the most abundant post-transcriptional RNA modification occurring both on adenine (m6A) and cytosine (m5C). m5C is an important modification with diverse roles such as regulating stress responses, stem cell proliferation and RNA metabolism. Here, we use RNA bisulfite sequencing (bsRNA-seq) for transcriptome-wide quantitative mapping of m5C in the model plant, Arabidopsis thaliana. We discover more than three thousand m5C sites in Arabidopsis mRNAs, long non-coding RNAs and other non-coding RNAs across three tissue types; siliques, seedling shoots and roots, and validate a number of these sites. Quantitative differences in methylated sites between these three tissues suggest tissue-specific regulation of m5C. Perturbing the RNA m5C methyltransferase TRM4B resulted in loss of m5C sites on mRNAs and non-coding RNAs and reduced the stability of tRNAAsp(GTC). We also demonstrate the importance of m5C in plant development as trm4b mutants show increased sensitivity to oxidative stress. Finally, we provide the first insights into the targeting mechanism of TRM4B by demonstrating that a 50nt sequence flanking m5C C3349 in MAG5 mRNA is sufficient to confer methylation of a transgene reporter in Nicotiana benthamiana.

FOXO3 LONGEVITY GENE FACTORY AT 6q21

Morris B.J.1, 2, 3, Donlon T.A. 1, 3, Chen R.1, Masaki K.1, 3, Allsopp R.C. 4, Wilcox D.C.1, 2, 3, Elliott A.1 and Willcox B. J. 1, 2

1Department of Research, Honolulu Heart Program/Honolulu-Asia Aging Study, Kuakini Medical Center, Honolulu, Hawaii, USA. 2Department of Geriatric Medicine, John A. Burns School of Medicine, University of Hawaii, Honolulu, Hawaii, USA. 3Department of Basic & Clinical Genomics Laboratory, School of Medical Sciences and Bosch Institute, University of Sydney, New South Wales, Australia. 4John A. Burns School of Medicine, University of Hawaii Manoa, Honolulu, Hawaii, USA.

The transcription factor FoxO3 regulates multiple genes involved in cell resilience. We identified 110 FOXO3 SNPs, 41 of which were associated with longevity. Thirteen were at binding sites for 18 transcription factors. By physical contact, via RNA polymerase II binding chromatin looping, with sites in the FOXO3 promoter, these likely function together as a cis-regulatory unit. FOXO3 was located at the centre of a 7.3 Mb 46-gene chromatin domain flanked by gene deserts. FOXO3 made long-range physical contacts via CCCTC-binding zinc finger protein (CTCF) binding sites with these 46 genes. The ‘archipelago’ of neighbourhood genes had a similar repertoire of functions as FoxO3, including stress resistance, nutrient sensing, cell proliferation, autophagy, apoptosis and stem cell maintenance. FOXO3 serves as the hub for an ‘interactome’ involved in healthy aging in those with favourable genotypes. Cellular stress stimulated FoxO3 expression in 20 lymphoblastoid cell lines, being 3-fold stronger for those with a favourable FOXO3 genotype. In FISH experiments, stress-induced activation of FOXO3 caused it to move towards neighbouring genes. In conclusion, we have shown, for the first time, that FOXO3 is at the central hub of a gene network on chromosome 6 involved in healthy aging. The findings represent an example of indirect contributions heralded in the new ‘omnigenic’ model linking genetic variation to complex polygenic conditions.

POSTERS

CURCUMIN INHIBITS VASCULOGENIC MIMICRY IN MESOTHELIOMA

Hocking A.J.1, Shashikanth M.2, Michael M.3 and Klebe S.1

1Department of Anatomical Pathology, Flinders Medical Centre, South Australia. 2Flinders Genomics Facility, School of Medicine, Flinders Medical Centre, South Australia. 3Department of Gastroenterology and Hepatology, Flinders Centre for Innovation in Cancer, Flinders Medical Centre, South Australia.

CANCELLED

Page 121

ComBio2017 • Adelaide, South Australia • 2–5 October, 2017
POS-WED-121
THE RNA 5'METHYLCYTOSINE METHYLTRANSFERASE NOP2 IS REQUIRED FOR OVULE DEVELOPMENT IN ARABIDOPSIS
Nguyen V., Searle I., Rakesh D. and Burgess A.
The University of Adelaide.

NOP2, a putative RNA 5-methylcytosine methyltransferase, is essential for ovule development in Arabidopsis thaliana. RNA has co-evolved with numerous post-transcriptional modifications to sculpt interactions with proteins and other molecules. RNA modifications are important for normal growth and development in plants and animals, for example mutations in NSUNS2/TRM4 in humans leads to microcephaly, short stature and neurological disorders. The RNA modification 5-methylcytosine (m5C) is an abundant modification on non-coding ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), long non-coding RNAs (lncRNAs) and messenger RNAs (mRNAs, Burgess et al., 2015 and David et al., 2017). In Arabidopsis thaliana we recently described more than 3,000 m5C sites across all classes of RNAs in root, leaf and silique tissues (Burgess et al., 2016, David et al., 2017 & Jun et al., unpublished). Here we describe the function of the evolutionary conserved m5C rRNA methyltransferase NOP2 in the model plant Arabidopsis thaliana. We bioinformatically identified three homologues of NOP2 in the Arabidopsis genome and named them, NOP2A, NOP2B and NOP2C. Previously, nop2a/dli2 mutants were described with reduced cell division in young leaves (Fujikura et al., 2009) however the molecular basis was not known. We identified loss of function nop2b and nop2c T-DNA mutants and observed no obvious morphological phenotypes. We tested for genetic redundancy by generating double mutants and demonstrated that nop2a nop2b mutants were lethal and abort at the two to eight nuclei stage during ovule development. As complete loss-of-function double mutants were lethal, we generated knockdown double mutants using an ARABIDOPSIS

POS-WED-123
CANCELLED

POS-WED-122
INTEGRATIVE ANALYSIS OF MIRNA AND MRNA EXPRESSION PROFILES IN COLORECTAL CANCER CELL RESPONSE TO METFORMIN
Orang A.V., McKinnon R.A., Petersen J., Sykes P.J. and Michael M.Z.
Flinders Centre for Innovation in Cancer, Flinders University, Flinders Medical Centre, Adelaide, South Australia 5042.

Since colorectal cancer (CRC) is the third most prevalent cancer in the world, the search for effective therapies is of vital importance. Metformin, a first-line diabetes drug linked to cancer prevention in retrospective clinical studies, inhibits cellular transformation and selectively represses cancer progression. MicroRNAs (miRNAs) are small non-coding RNAs involved in most cellular processes, including cancer cell metabolism. Although several metabolic effects of metformin treatment have been investigated in different cancers, detailed analysis of the resultant changes in gene expression is still required. In this current study HCT116 cells were treated with different metformin doses for 3 days. We then performed RNA-seq and small RNA-seq next-generation sequencing (Illumina) to identify the differentially expressed (DE) miRNA and mRNA profiles that result following 2.5 mM Metformin treatment. Subsequently, bioinformatic analyses were undertaken that included miRNA target prediction, molecular pathway and network analyses of integrated DE miRNA and mRNA datasets. As expected, metformin treatment resulted in a significant reduction in HCT116 cell growth, ATP synthesis, Oxygen Consumption Rate (OCR) and an increase in Extracellular Acidification Rate (ECAR) in a dose-dependent manner. A threshold effect was observed for the ability of metformin to modulate metabolic pathways. Reverse transcription polymerase chain reaction (RT-PCR) analysis of a range of DE miRNAs and mRNAs validated sequencing results. MicroRNA and mRNA expression profiles were integrated to identify significant miRNA-mRNA correlations and potential regulatory networks. The identified networks will ultimately be tested using functional genomics screens in a systems biology approach.

POS-WED-124
CHANGES IN THE EPIGENOME AND TRANSCRIPTOME OF BARLEY UNDER SALINITY STRESS
Smith J.L.P.1, Rodriguez Lopez C.M.2, Shavrukov Y.N.1, 3, Michael M.Z.4 and Anderson P.A.1
1Flinders University, College of Science and Engineering, Sturt Road, Bedford Park, SA 5042, Australia. 2Environmental Epigenetics and Genetics Group, University of Adelaide, Waite Campus, PMB1 Glen Osmond, SA, 5064, Australia. 3Adelaide University, School of Agriculture, Food and Wine, Waite Campus, Glen Osmond, SA 5064, Australia. 4Flinders Centre for Innovation in Cancer, Flinders Drive, Bedford Park, SA 5042, Australia.

Epigenetic mechanisms are potentially heritable molecular changes that affect gene expression, leading to differences in phenotype without changing the DNA sequence of the organism. In plants, such mechanisms are involved in the control of a range of processes, including response to stress. DNA methylation is an epigenetic mechanism used by various organisms to adapt to changing environmental conditions by altering localised accessibility of the genome to transcription factors, ultimately affecting gene expression levels. Stress induced de-novo DNA methylation in plants is organ specific and can be guided by a class of short interfering RNA, typically 24-nt long. This project uses epiGBS, a reduced representation genome bisulphite sequencing method, coupled with small RNA and whole transcriptome Next Generation Sequencing to investigate interactions between short interfering RNAs, DNA methylation and gene expression in the leaves and roots of barley under salt stress. The bioinformatics methods developed link sequence information from DNA methylation, small RNA and the transcriptome and lead to a greater understanding of how this important crop deals with salinity stress.
POS-WED-125

NEXT GENERATION SEQUENCING ANALYSIS OF 51 GENES OF INTEREST IN BRCA1 AND BRCA2 MUTATION NEGATIVE INDIVIDUALS WITH A FAMILIAL HISTORY OF BREAST CANCER

Thompson-Peach C.A.L.1, Michael M.Z.2, Grist S.A.3, Kuss B.J.1,3 and Lower K.M.1

1Department of Molecular Medicine and Pathology, Flinders University, Bedford Park, South Australia, Australia. 2Flinders Centre for Innovation in Cancer, Flinders Medical Centre, Flinders University, Bedford Park, South Australia, Australia. 3Department of Molecular Pathology, SA Pathology, Bedford Park, South Australia, Australia.

Breast cancer is the most common cancer affecting Australian women, with a prevalence of 1 in 8 women diagnosed by age 85. Although a majority of these cases are attributed to sporadic cancers, epidemiology and genetic studies have identified familial history as a strong risk factor for the development of breast and ovarian cancer. It has been demonstrated that inherited mutations in the main breast cancer susceptibility genes, BRCA1 and BRCA2, account for approximately 20% familial breast cancer cases. Several other predisposition genes have been identified; however the underlying cause of more than 70% of familial breast cancer cases is still unknown. This suggests that more breast cancer susceptibility genes exist. BRCA1/2 play central roles in the maintenance of genomic integrity and stability through their roles in the DNA repair pathway and cell cycle checkpoint control. Therefore, it is biologically feasible that mutations within genes which directly interact with BRCA1/2 or DNA damage repair and checkpoint control pathways play a role in predisposing families to inherited breast cancer. Through the use of targeted gene capture and next generation sequencing (NGS), it is possible to sequence a number of genes known to function in these pathways. When undertaken on a cohort of individuals with BRCA1/2 negative familial breast cancer, this method is potentially able to identify the genetic cause of the cancer in these families. For this research, a comprehensive gene panel was developed based on literature and bioinformatic analysis of DNA repair and checkpoint control pathways play a role in predisposing families to inherited breast cancer. Through the use of targeted gene capture and next generation sequencing (NGS), it is possible to sequence a number of genes known to function in these pathways. When undertaken on a cohort of individuals with BRCA1/2 negative familial breast cancer, this method is potentially able to identify the genetic cause of the cancer in these families. This result suggests that the QTL controls biomass accumulation at early plant growth stage contributes to maintain large number of fertile tillers and grain yield under terminal drought and heat stress conditions and those of South Australian climate. The yield QTL interval was fine mapped to 2 cM, corresponding to a 6 Mbp sequence. Hundred thirty one predicted genes were identified in this region and will be studied to identify a new yield gene in wheat.

POS-WED-127

THE EFFECTS OF 5-METHYLCYTOSINE ON THE STABILITY OF tRNAs IN ARABIDOPSIS THALIANA

Zhao J., Burgess A., David R. and Searle I.
The University of Adelaide, Adelaide, SA, Australia.

Abstract: More than 150 post-transcriptional RNA modifications have been described on transfer RNAs (tRNAs) in plants and animals. One abundant RNA modification is 5-methylcytosine (5mC) and emerging evidence is demonstrating m5C plays key regulatory functions on tRNAs and other cellular RNAs (Burgess et al., 2016, David et al., 2017). To explore the landscape and function of m5C on tRNAs in plants, we used: bisulphite conversion and strand specific RNA Illumina sequencing (bs-RNAseq), identified mutants in several m5C RNA methyltransferases, and characterization these mutants at the molecular and phenotypic levels. We identified more than 39 highly methylated sites in predicted structural positions of only nuclear tRNAs but not chloroplast and other cellular RNAs (Burgess et al., 2017). To explore the landscape and function of m5C on tRNAs in plants, we used: bisulphite conversion and strand specific RNA Illumina sequencing (bs-RNAseq), identified mutants in several m5C RNA methyltransferases, and characterization these mutants at the molecular and phenotypic levels. We identified more than 39 highly methylated sites in predicted structural positions of only nuclear tRNAs but not chloroplast and other cellular RNAs (Burgess et al., 2017). We also demonstrated by using bs-RNAseq on tRNAs from the single-celled algae Nannochloropsis oculata, the macro algae Caulerpa taxifolia and multi-cellular higher plants Brassica rapa, Triticum durum and Ginkgo biloba that these methylated nuclear tRNA sites were mostly conserved in position and present methylation through plant evolution. In Arabidopsis, we demonstrated that two methylated tRNAs, tRNA^{Asp} and tRNA^{Thr}, are cleaved at a structural loop under oxidative stress. We also demonstrated that this ribonuclease-mediated tRNA cleavage is increased in the m5C methyltransferase mutant trm4d. We are currently characterizing five candidate ribonucleases, ARIK1-5, to identify the key mediator or tRNA cleavage. Functional analysis of the tRNA-fragments will also be presented.

POS-WED-128

MICRORNA-155-5P REGULATES DENGUE VIRUS INDUCED INFLAMMATION IN ENDOTHELIAL CELLS

Abraham A.M.1, Aloia A.L.2, Cabezas S.1 and Carr J.M.1
1Microbiology and Infectious Diseases, School of Medicine, Flinders University, Bedford Park, Adelaide, SA 5042, Australia. 2Cell Screen SA, Flinders Centre for Innovation in Cancer, Flinders Drive, Bedford Park, SA 5042, Australia.

Dengue virus (DENV) infection of cells including macrophages and endothelial cells (EC) result in release of inflammatory and innate factors. Many of these factors are vasoactive and can act on EC lining the vasculature to alter endothelial barrier function that may be manifested as vascular leak syndrome as observed in patients with severe dengue. Cellular microRNAs (miRNA) that function to regulate gene expression by cleaving mRNA or through translational repression have a role in regulating innate inflammatory responses and vascular function. Here we explore the role of the miRNA miR-55-5p (known to regulate factors associated with inflammation) in the context of DENV infection. Our results show that in DENV infected macrophages and human umbilical vein endothelial cells (HUVEC), the levels of miR-155-5p increases. In HUVECs, overexpression of miR-155-5p using miR-155-5p mimics did not alter DENV replication or virus release in infected cells but DENV release was diminished in miR-155-5p inhibitor treated HUVEC. Further miR-155-5p mimics decreased DENV-induced miRNA for TNF-α, IFN-β, ICAM (cell adhesion molecule) and the interferon stimulated gene viperin. In contrast, miR155-5p inhibitors augmented DENV induced TNF-α and IFN-β transcripts, although no significant change in ICAM or viperin was observed. Further studies are underway to confirm these observations and extend the investigation to macrophages. Our studies may define novel miRNA therapeutic to treat DENV-induced vascular leak by targeting exacerbated inflammation in EC.
Everyday billions of cells undergo apoptosis in the human body and rapid removal is essential to avoid the onset of inflammatory disease. It has been suggested that the generation of vesicles termed apoptotic bodies (ApoBDs) could mediate apoptotic cell clearance by forming small, bite-sized pieces that could be easily engulfed by surrounding phagocytes. ApoBDS are generated through a three-step process known as apoptotic cell disassembly. Recently, we demonstrated that monocytes undergo a highly coordinated disassembly pathway giving rise to long, beaded membrane protrusions (coined beaded apopodia) that fragment to release ApoBDS. As the mechanism underlying beaded apopodia formation is unclear, we performed proteomic analysis of THP1 monocyte ApoBDS and found that the protein Plexin B2 was enriched in the ApoBD fraction. Additionally, western blot analysis demonstrated that Plexin B2 undergoes apoptotic processing by caspases. Therefore, to determine if Plexin B2 is involved in this process, we performed THP1-Plexin B2-/- cells. Differential interference contrast microscopy analysis demonstrated that depletion of Plexin B2 significantly impaired the level of beaded apopodia formation and subsequent fragmentation into ApoBDS. Importantly, expression of the Plexin B2 beta-domain in the depleted cells restored beaded apopodia and ApoBD generation. Finally, to assess the functional significance of beaded apopodia formation we performed a series of engulfment assays which demonstrated that depletion of Plexin B2 significantly impaired the engulfment efficiency in vitro and in vivo. Altogether, these findings have identified the first positive regulator of apoptotic cell disassembly and clearly demonstrate the importance of this process in mediating efficient apoptotic cell clearance.

The effect of epidermal growth factor on myosin expression in submandibular salivary glands of rats treated with botulinum toxin

Botulinum toxin (BTX) has a number of cosmetic, medical and dental applications. One of the dental applications of BTX is treatment of excessive salivation. Local BTX injection into salivary glands has an effect on the histology, and integrity of the tissues. Myosins are contractile proteins that are highly expressed in myoepithelial cells (MECs) and present around salivary gland ducts and acini to help maintain normal salivary flow. The aim of this study is to investigate the effect of BTX injections on the submandibular salivary glands of adult female Albino rats, when administered solely or in conjunction with Epidermal growth factor (EGF) through immunohistochemical localization of myosin in the parenchyma of the gland. Sixty rats were used in this study and were equally divided into control (saline) group, BTX group and EGF + BTX group (Combined treatment). The results obtained from this study showed that myosin expression in submandibular salivary glands of rats significantly decreased after a single subcutaneous injection of 2.5 units of BTX in 0.1ml saline. However, daily intra-peritoneal injections of EGF with a dose of 10 μg/Kg body weight restored normal levels of myosin expression, as well as normal integrity and function of submandibular salivary glands. Further confirmation of the above findings is recommended through immunohistochemical localization of E-cadherin as well as ultrastructural examination of submandibular salivary glands treated with BTX and EGF.
POSTERS

POS-WED-133
ANALYSIS OF TRANSCRIPTION FACTOR SIM2 VARIANTS PRESENT IN PATIENTS WITH INTELLECTUAL DISABILITIES

Button E.L., Bersten D.C. and Whitelaw M.L.
Department of Molecular and Cellular Biology, University of Adelaide, South Australia.

Single-Minded 2 (SIM2) is a member of the basic-helix-loop-helix/PER-ARNT-SIM (bHLH/PAS) family of transcription factors, which are known to play important roles in biological processes such as development, oxygen homeostasis and stress responses. SIM2 mRNA is expressed within the brain both during development and postnatally, however the function of SIM2 within the brain is not well characterised. SIM2 knockout (KO) mice die perinatally, displaying varying phenotypes including dysmorphologies such as cleft palates and sporadic scoliosis. These mice also display a reduced number of somatostatin and thyrotropin releasing hormone expressing neurons within the hypothalamus. A screen was performed on an exome sequencing database to identify SIM2 variants in patients with intellectual disabilities, delayed development of speech, dysmorphic features and scoliosis. We performed reporter gene assays on a number of the variants and identified non-synonymous mutations that cause a change in transcriptional activity. These variants were then further characterised in order to determine the mechanism behind this change in activity. This study highlighted several SIM2 variants found in patients with disabilities and validated a candidate set as potential disease causing or contributing variants.

POS-WED-135
MOLECULAR AND EVOLUTIONARY CONSERVATION OF ABA AND BLUE LIGHT SIGNALLING IN STOMATAL REGULATION

Cai S.1,2, Chen G.2 and Chen Z.H.1
1School of Science and Health, Western Sydney University, Penrith, NSW, 2777, Australia. 2College of Agriculture and Biotechnology, Zhejiang University, Hangzhou 310058, China.

Evolutionary trajectories of land plants have led to structurally complex and functionally active stomata for terrestrial life. The unique morphology, development and molecular regulation of stomata enable their rapid environmental response. ABA-driven stomatal regulation reportedly evolved after the divergence of mosses and ferns. Here, we observed that a number of ABA signaling, membrane transporter, and ABA receptor protein families diversified over the evolutionary history of land plants. Phylogenetic analysis of the key ABA signaling proteins, including ABA-induced stomatal closure, has been found in mosses, ferns, gymnosperms, and angiosperms. Evolutionary conservation of stomatal response to ABA is observed over the angiosperm family. Comparative transcriptomic analysis has identified a suite of ABA responsive differentially expressed genes encoding proteins associated with ABA biosynthesis, transport, reception, transcription, signaling, and ion and sugar transport, which fit the general ABA signaling pathway constructed from Arabidopsis and barley. Furthermore, our stomatal assays have ABA-induced stomatal closure and blue and UV-A light-induced stomatal opening on epidermal peels of a range of fern species. Stomata of fern species are more responsive to blue and UV-A light conditions as compared to other species. Understanding the evolutionary trends of stomatal regulation will inform functional manipulation of plant productivity and water use and will benefit future efforts towards food security and ecological diversity.

POS-WED-136
THE EFFECTS OF UV EXPOSURE ON CORNEAL EPITHELIAL REGENERATION AND MAINTENANCE

Delic N.C.1,2, Lobo E.P.3, Di Girolamo N.4, Halliday G.M.2 and Lyons J.G.1,2,5
1Discipline of Dermatology, Bosch Institute and Living Healthier Lives Under the Australian Sun project node of the Charles Perkins Centre, University of Sydney, NSW 2006, Australia. 2Immune Imaging Program, Centenary Institute for Cancer Medicine and Cell Biology, NSW 2050, Australia. 3School of Mathematics and Statistics, University of Sydney, NSW 2006, Australia. 4School of Medical Sciences, University of New South Wales, NSW 2052, Australia. 5Sydney Head and Neck Cancer Institute, Cancer Services, Ro Aly Prince Alfred Hospital, NSW 2050, Australia.

The primary cause of skin and ocular carcinomas is the ultraviolet radiation (UVR) emitted from sunlight. After the skin, the eyes are the organs that are most highly exposed to light. Specifically, the ability of the cornea to self-repair and maintain clarity is essential, as any deviations result in vision loss and neoplasia. The corneal epithelium is derived from asymmetrically dividing stem cells that predominantly reside in the limbus. The limbus is found at the corneoconjunctival junction. We have previously shown that, during homeostasis, the corneal epithelial cells of adult mice form spoke-like clonal growth patterns, moving from the limbus towards the centre of the cornea. We have now shown that the rate of clonal growth from the limbus to the central cornea is increased when exposed to UVR. TUNEL assays have revealed that 24h-post UVR, there was only a 3% increase in apoptotic cells. Conversely, preliminary EdC-staining showed that there is an increase in proliferation at the periphery of the cornea, decreasing closer to the centre, 24h-post UVR. The biological data are in agreement with a mathematical model that shows that the proliferative potential of the corneal epithelial cells must be reduced in order for the radial pattern to be maintained after UVR exposure. However, the molecular mechanism required to signal the decrease in proliferative potential remains unknown. The increase in migration and low rate of apoptosis suggests that another mechanism must be being used in order to remove damaged cells and mobilize cells from the limbus to maintain corneal thickness.

POS-WED-134
RETROMER MODULATES AUTOPIHY

Carosi J.M.1,2, Teasdale R.3 and Sargeant T.J.1
1Neurobiology Section, Lysosomal Diseases Research Unit, Nutrition & Metabolism Theme, SAHMRI. 2University of South Australia. 3Institute for Molecular Bioscience, University of Queensland.

Retromer is an endosomal coat-complex which regulates the sorting and trafficking of various receptors to prevent their lysosomal degradation. Cargo specificity and selection on the endosomal membrane by retromer is typically carried out by its sub-complex consisting of VPS26-VPS35-VPS29. Retromer controls the endosome-to-Golgi (retrograde) and endosome-to-cell surface (recycling) trafficking of unique receptors, processes that are impaired in Alzheimer’s disease due to VPS35 down-regulation. Here, we show that genetic deletion of VPS35 in HeLa cells causes a block in the resolution of autophagy, indicated by an increase in the level of the autophagy marker LC3-II, detected by Western blot. Interestingly, this block is not due to perturbed lysosomal function as the proteolytic activities of cathepsins B/L and -D/E are unaltered in vitro. Next, we found that VPS35-deficient cells do not respond to pharmacological inducers of autophagy that inhibit mTORC1, suggesting that VPS35 deficiency also enhances the induction of autophagy. Taken together, these data reveal insights into how retromer dysfunction may contribute, at least in part, to the altered autophagic flux that is observed in Alzheimer’s disease.
DUAL ROLE OF P120CTN IN CANCER: EPITHELIAL VS MESENCHYMAL
Fonseka P., Atukorala I., Liem M. and Mathivanan S.
La Trobe University.

Neuroblastoma, a paediatric cancer, accounts for 15% of childhood cancer mortality. The exact mechanisms by which this aggressive cell type resists treatment is poorly understood. Here, we hypothesise that neuroblastoma cells have high expression of mesenchymal markers and hence could attribute to the aggressive phenotype. P120ctn is downregulated in epithelial cancers and is known to play a major role in EMT and aggressiveness. In this study, immunohistochemical staining of neuroblastoma patient tissues suggested that p120ctn is highly abundant. Hence, the role of p120ctn and N-Myc in neuroblastoma aggressiveness was investigated by using RNA interference. Amplification of N-Myc oncogene occurs in 20% of neuroblastoma patients and is considered high risk as it correlates with aggressiveness and poor prognosis. Interestingly, knockdown of p120ctn downregulated N-Myc both at mRNA and protein levels. Upon knockdown of p120ctn and N-Myc, the proliferation, invasion and migration of neuroblastoma cells were significantly reduced. Quantitative proteomic and qPCR analysis of cells revealed that p120ctn knockdown cells underwent mesenchymal-to-epithelial transition. Confocal microscopy and subcellular fractionation showed nuclear accumulation of β-catenin upon p120ctn knockdown. Once in the nucleus, β-catenin activated Wnt signalling pathway and upregulated Wnt target genes. Interestingly, downregulation of p120ctn sensitised the neuroblastoma cells to doxorubicin. Currently, there is no published study that explores the role of p120ctn in neuroblastoma. However, these findings are contradictory to scientific literature in the context of the functional role of p120ctn in epithelial cancer. Hence to validate our findings, we established knockdown of p120ctn in epithelial colorectal cancer cells. Consistent with the literature, knockdown of p120ctn induced EMT, proliferation and migration. These results suggest that the role of p120ctn is cell type dependent. Overall, the findings from this study suggest that p120ctn plays a pivotal role in progression of neuroblastoma.

INVESTIGATING MECHANISMS AND BIOLOGICAL FUNCTIONS OF THE OBESITY RELATED TRANSCRIPTION FACTOR SINGLE-MINDED 1
Department of Molecular and Cellular Biology, University of Adelaide, South Australia.

The basic helix-loop-helix/Per-Arnt-Sim (bHLH/PAS) transcription factor Single Minded 1 (SIM1) has essential roles in the development and function of hypothalamic cell lineages. Haploinsufficiency of Sim1 in mice results in increased weight gain due to hyperphagia and increased linear growth. We have recently established that non-synonymous point mutants of SIM1 can underpin a monogenic cause of obesity in humans. Several studies indicate that SIM1 plays an important role in the appetite controlling Leptin-Melanocortin signalling pathway in the hypothalamus, however the target genes of SIM1 and its up- and downstream regulatory pathways have yet to be defined. To further our understanding of the role of SIM1 in appetite control, we have generated a transgenic mouse model with GFP under the control of the Sim1 promoter composite with a weakly functioning mutant of SIM1. This mouse model, in combination with cell based assays, will be used to identify novel target genes and investigate mechanisms of regulation of SIM1.
**POS-WED-141**

**SEX DIFFERENCES IN A RAT MODEL OF CORNEAL NEOVASCULARIZATION**

Iriani Y.D.¹, Pulford E.², Mortimer L.A.³, Klebe S.³ and Williams K.A.¹

¹Department of Ophthalmology, Flinders University, South Australia. ²Department of Anatomical Pathology, Flinders University, South Australia.

Sex-based differences in prevalence have been reported for some human ocular diseases, including diabetic retinopathy and age-related macular degeneration. We investigated whether sex-based differences occurred in corneal neovascularization in the rat. Corneal neovascularization was induced in adult male and female outbred albino Sprague-Dawley, outbred pigmented Hooded Wistar, inbred albino Fischer 344 and inbred pigmented Dark Agouti rats following superficial cautery with silver nitrate. Rats were euthanised 14 days post-cautery, blood vessels perfused with haematoxylin, the corneas flattened, imaged and neovascularization quantified by a masked observer using ImageJ (NIH).

Sternal cell test was applied to mean neovascular area. The expression of androgen and estrogen receptors was assessed in normal and cauterised Sprague-Dawley corneas by immunohistochemistry. Male rats from all strains developed more oedema than females and exhibited significantly greater corneal neovascular area. Sprague-Dawley males 43±12% n=8, females 25±5% n=12, p<0.001; Hooded Wistar males 32±6% n=8, females 22±5% n=12, p=0.001; Fischer 344 males 38±10% n=9, females 27±8% n=8, p=0.028; Dark Agouti males 37±11% n=9, females 26±7% n=9, p=0.022. Androgen receptor was expressed in the corneal epithelium in males and females under normal and pathological conditions. Estrogen receptor was expressed in the corneal epithelium of females under normal and pathological conditions, and in males under pathological conditions. However, vascular endothelial cells expressed neither androgen nor estrogen receptors. Corneal neovascular area was significantly greater in male than female animals, irrespective of strain or pigmentation, but was unrelated to expression of sex hormone receptors in the cornea. These data suggest that preclinical animal models should incorporate male and female animals to account for sex-based differences.

**POS-WED-143**

**ACTIN REGULATION IN AMYTOTROPHIC LATERAL SCLEROSIS (ALS)**

Jagaraj C.J.¹, Sundaramoorthy V.¹, Walker A.¹ and Atkin J.², ³

¹Department of Biomedical Science, Sydney, Macquarie University. ²Department of Biochemistry and Genetics, VIC, Latrobe University.

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disorder, characterised by the specific loss of motor neurons in the brain, brain stem and spinal cord. The cause of ALS is unknown in 90 to 95% cases (sporadic ALS) whereas 5-10% cases are inherited (Familial ALS). Mutations in the C9orf72 gene account for the majority of familial cases of ALS and FTD (~50%). The pathological hallmark of ALS is the formation of intracellular cytoplasmic inclusions, containing misfolded proteins. Disturbance to protein folding, autophagy, the ubiquitin-proteasome system (UPS) dysfunction, RNA dysfunction and DNA damage, are implicated as possible disease mechanisms in ALS, but the aetiology remains unclear. Recent evidence suggests that actin dynamics is modulated in motor neurons. Actin polymerisation/depolymerisation are dynamic processes in neurons that are essential for neuronal motility, synaptic vesicle formation and transport. Hence, in this study, we examined actin regulation in C9orf72-positive and sporadic ALS cases. Our results reveal that C9orf72 protein acts as a regulator of actin dynamics. Similarly, fibrolast from C9orf72-positiveALS patients display increased levels of F-actin compared to control fibroblasts, suggesting that actin is polymerised more in C9orf72-patient fibroblasts. Our data also suggest that actin is polymerised more in sporadic ALS patient spinal cord tissues compared to control tissues. In addition, cofilin (Actin depolymerising factor) is phosphorylated in sporadic ALS spinal cord tissues. This suggest cofilin is inactive in ALS and therefore actin is polymerised more in ALS. Together, our results indicate that actin regulation is dysregulated as a potentially new pathogenic mechanism in both C9orf72-ALS and sporadic ALS.

**POS-WED-144**

**A PARADIGM IN IMMUNOCHEMISTRY: DEMONSTRATING MONOCLONAL ANTIBODIES TO SPATIALLY DISTINCT EPITOPES ON SYNTENIN-1**

Johnson I.R.D.¹, Heattie J.K.², Moore C.R.², Sorvina A.³, Logan J.³, Todd C.T.², Parkinson-Lawrence E.J.², O’Leary J.³, Butler L.M.² and Brooks D.A.¹

¹University of South Australia. ²University of Adelaide. ³Trinity College Dublin.

Syntenin-1 plays a critical role in multiple cellular functions, including the regulation of membrane trafficking, cell adhesion, exosome biogenesis and transcription, with implications in cancer cell growth, migration and metastasis. This suggests that Syntenin-1 is a critical factor in cancer progression. Consequently, this membrane-associated protein might be expected to have different cellular distributions based on specific functional interactions. To overcome potential limitations of commercial antibodies we sought to develop specific monoclonal antibodies to syntenin-1 that had no other potential cross interactions at the six-amino acid level. We have investigated the specificity of two such monoclonal antibodies to spatially distinct epitopes on syntenin-1, in the context of its different functional cell biology. Antibody specificity was confirmed by Western blotting, two-site ELISA and protein knockdown. Our 3A11-1 monoclonal antibody produced a fibrilla staining pattern consistent with a cytoskeleton distribution. In contrast, the 2C6-2 antibody produced a punctate vesicular staining pattern that was consistent with staining of intracellular compartments such as endosomes. Nododazol, affected Syntenin-3A11 distribution, suggesting its association with microtubules. Cytochalasin D showed patterns of staining similar to untreated cells. This is the first time that Syntenin-1 has been observed in this pattern with such clarity. Protein modelling indicates that both epitopes are expected to have monoclonal antibodies that are specific to the microtubule-binding region of Syntenin-1. 2C6-2 may be spatially restricted when Syntenin-1 is localised to microtubules; an absence of microtubule association could be expected in more punctate staining. We postulate that the wide functionally of Syntenin-1 confers significant conformational change and masking of epitopes that may not be fully realised by polyclonal antibodies.

**POS-WED-142**

**CI NEGATIVE AUTOREGULATION OF THE 186 BACTERIOPHAGE LYSOGENIC PROMOTER AND THE ROLE OF THIS REGULATION IN PROPHAGE INDUCTION**

Isabel A., Dodd I. and Shearin K.

The University of Adelaide, Adelaide, South Australia, 5005, Australia.

The SOS inducible temperate coliphage 186 has the ability to efficiently transition between two alternative modes of development; lysis or lysogeny. During lysogeny, 186 integrates its genome into the host bacterium's chromosome. 186 maintains its lysogenic lifecycle via the CI immunity repressor, which represses both the lytic (pR) and lysogenic (pL) promoters. Regulation of pL generates a negative feedback autoregulatory loop that produces CI levels in a lysogen sufficient to maintain lysogeny and yet still allows the phage to undergo prophage induction (the event in which 186 switches from lysogenic to lytic development). Studies have shown that disrupting the spacing between CI operator sites at pR and pL has drastic detrimental effects on prophage induction. Using a series of chromosomally integrated CI expression/reporter systems, we showed how disrupting the 63bp spacing between pR and pL CI operator sites with a 5bp deletion (the goa8 mutation) significantly disrupts CI autoregulation, which in turn compromises 186 prophage induction. Western blot analysis of a 186goa8 lysogen further showed that this lysogen generates 1.65-fold more CI than a 186 lysogen. It appears that the ability of CI to form a negative feedback loop (is not only important to sustain CI levels at an optimum single steady state to maintain lysogeny) is required to conserve the ability of 186 to rapidly switch from lysogenic to lytic development. Furthermore, given the goa8 mutation only increases CI levels by 1.65-fold demonstrates how fine-tuned the 186 switch system is and how critical regulatory proteins must remain at their optimum steady states to allow for maximal switch efficiency.
POS-WED-145

INVESTIGATING THE MECHANISM OF ANTISENSE REGULATION OF THE LATE GENE ACTIVATOR Q IN BACTERIOPHAGE LAMBDA


Bacteriophage lambda has proven to be an important model organism in many aspects of molecular biology, including the mechanistic understanding of key developmental decisions. In the lambda genetic switch between lytic and lysogenic development, the CI protein is a crucial determinant that promotes development towards the lysogenic state. CI is known to be a activator of paQ; an antisense promoter located within the coding sequence of the late gene activator Q. CI expression leads to the down-regulation of Q expression. Since a delay in Q activity during the initial stages of decision making has been shown to be important in establishing lysogeny, determining the currently unknown mechanism by which paQ negatively regulates Q activity may help to improve current models for lysogenic development. Here we propose methods to test the two main hypotheses for Q regulation by paQ: 1) cis-acting transcriptional interference between divergent promoters pR and paQ or 2) trans-acting antisense inhibition between Q and paQ transcripts. We aim to use integrated LacZ reporter constructs in conjunction with an inducible CII expression system to measure the amount of transcriptional interference from paQ in vivo. Initially, we aim to characterise the promoter strength of paQ and determine the efficiency of its postulated terminator (taQ). Additionally, we aim to express the paQ transcript in trans to determine whether it can rescue the clear plaque phenotype observed in lambda carrying a mutated paQ promoter and hence show evidence of sense-antisense interaction. Thus, we aim to determine the mechanism by which CI mediated down-regulation of Q activity occurs via its antisense promoter; paQ.

POS-WED-147

MICRORNA-342 A NOVEL SUPPRESSOR OF A PRO-METASTATIC GENE NETWORK IN TRIPLE-NEGATIVE BREAST CANCER

Lumb R.1, Arnet V.K.1,2, Johnstone C.N.3, Roslan S.1, Li X.1, Phillips C.A.1, Khew-Goodall Y.1, Goodall G.J.1,2, Anderson R.L.1,2 and Gregory P.A.1,2

1Centre for Cancer Biology – An Alliance between SA Pathology and the University of South Australia, Adelaide, SA, Australia. 2Discipline of Medicine, The University of Adelaide, Adelaide, SA, Australia. 3Olivia Newton-John Cancer Research Institute, Heidelberg Vic Australia.

Breast cancer is the most frequently diagnosed cancer in women and the second leading cause of female cancer related death. Despite significant advances in early detection, surgery and therapy, treatment remains a challenge if metastatic disease develops. Metastasis occurs with high frequency in triple negative (ER/PR/HER2 negative, TNBC) breast cancers, which are a heterogeneous group of cancers with poor clinical outcome. To date, there are no there are no approved targeted therapies for TNBC, and thus standard treatments are combinations of chemotherapy agents that display mixed clinical responses. It is clear that new prognostic and therapeutic targets are required to stratify and treat TNBC, particularly the subtypes that exhibit higher rates of metastasis. We used an integrated approach to identify miRNAs that influence breast cancer metastasis as well as indicate patient outcome, specifically in TNBC. Through this we identified miR-342 which we found to: (1) strongly downregulated in mouse and human TNBC cell lines that are prone to metastasis, (2) sufficient to repress breast cancer metastasis in immune competent and xenograft mouse models, and (3) an independent prognostic marker of patient outcome in large TNBC cohorts. We further identified that multiple 342 targets are members of two metastatic pathways including the E2F1- and CDC42-driven pathways, which may represent new targets for treatment of TNBC.

POS-WED-146

RMD-MEDIATED F-ACTIN ORGANIZATION CONTRIBUTES TO POLLEN TUBE GROWTH IN RICE

Li G.1, Yang X.J.1, Song Y.2 and Zhang D.B.1,2

1School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, Urrbrae, South Australia 5064, Australia. 2The University of Adelaide-Shanghai Jiao Tong University Joint Laboratory for Plant Science and Breeding, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, China.

For the successful fertilization in angiosperms, pollen tubes deliver sperms into the ovules via rapid tip growth. The actin-binding proteins (ABPs)-mediated organization of the actin cytoskeleton within the pollen tube plays a crucial role in this polarized process. However, the mechanism underlying the actin filaments (F-actin) array polarity and behaviors in pollen tube growth remains largely unknown. Here, we demonstrate that an actin-organizing protein Rice Morphology Determinant (RMD), a type II formin from rice (Oryza sativa), controls pollen tube growth by modulating F-actin cable direction, dynamics and branching. rmd mutant exhibits abnormal rice pollen tube growth and decreased pollen grain germination rate in vitro and in vivo. Apical dynamics and shank longitudinal cable direction of F-actin are altered in rmd mutant pollen tubes, indicating the novel role of RMD in F-actin polarity during tip growth. In addition, tobacco (Nicotiana tabacum) assays demonstrate that RMD localizes at the tip of pollen tube, which is essential for pollen tube growth and polarity as well as F-actin organization. Furthermore, RMD-guided F-actin array direction and dynamics positively regulate the deposition of cell wall components, the pattern of vesicle trafficking and the efficiency of cytoplasmic streaming during rice pollen tube growth. Collectively, our results suggest that a type II formin member RMD is essential for dynamic and spatial regulation in pollen tube growth via modulating F-actin organization and array orientation in model crop rice. This work provides insights into tip-focused cell growth and polarity.

POS-WED-148

IN-VIVO PROXIMITY-BASED BIOTINYLATION IDENTIFIES INTERSECTIN-1 AS AN IMPORTANT REGULATOR OF ACTIN DYNAMICS AT THE GOLGI

Makhoul C., Gosavi P., Duffield R., Williamson N. and Gleeson P.A.

Bio21, 30 Flemington Road, Parkville, Victoria, 3010.

In mammalian cells, the cis, medial and trans-Golgi compartments are organized into a series of flattened membrane stacks that are laterally connected. This forms a structure that is referred to as the Golgi ribbon, which is positioned in a pericentrosomal location within the cell. The maintenance of the Golgi ribbon relies on a dynamic balance between the actin and microtubule network as well as the interaction of Golgi-localized proteins with both of these networks. In a previous study, it was observed that the overexpression of a trans-Golgi network-targeted membrane tether, GCC88, lead to the loss of the Golgi ribbon. GCC88 belongs to a family of Golgi-localized proteins known as the GRIP domain-containing golpins. To investigate the role of GCC88 in the fragmentation of the Golgi ribbon, we used the proximity-based biotinylation method, BioID, to screen for candidate protein interactors that could be responsible for the GCC88 mediated loss of the Golgi ribbon. We identified Intersectin-1 (ITSN1), a guanine nucleotide exchange factor for cdc42, as an interactor of GCC88 and have shown that ITSN1 localizes to the Golgi. Treatment with actin depolymerizing drugs results in a reversal of the phenotype mediated by the overexpression of GCC88. We propose a model where GCC88 acts to recruit actin to the Golgi and allow for rapid fragmentation of the Golgi ribbon, through the overexpression of GCC88, is driven by an increase in actin polymerization at the Golgi.
**POS-WED-149**

**THE TRANSITION TO REGIONAL ENDOTHERMY IN PACIFIC BLUEFIN TUNA, THUNNUS ORIENTALIS**

Malik A.1, Dickson K.2, Kitagawa T.1, Fujioka K.1 and Schuller K.1

1Flinders University, Adelaide, South Australia. 2California State University Fullerton, USA.

The ability to maintain a body temperature higher than the surrounding environment by conserving metabolically-generated heat (endothermy) is a characteristic that is shared by birds and mammals. This ability is generally not associated with fishes. In Pacific bluefin tuna (PBT), vascular counter-current heat exchangers (retia) conserve metabolic heat, allowing the temperatures of the aerobic (red) locomotor muscle, viscera, and cranial region to be elevated above water temperature (regional endothermy). The transcriptional coactivator peroxisome proliferator-activated receptor-gamma coactivator-1 alpha (PGC-1α) is a master regulator of mitochondrial biogenesis and function in mammals. We hypothesize that the development of regional endothermy in fishes is associated with increasing gene expression of PGC-1α as well as its target transcription factors. In this study we analyzed the cranial, visceral and red muscle temperatures, activity of the mitochondrial enzyme citrate synthase (CS) and cytochrome c oxidase (COX) and gene expression of PGC-1α, CS and COX1 in the red and white muscle in year 0 bluefin (18.4-60.5 cm fork length, collected in August and November of 2016 and March 2017). The larger bluefin displayed significantly elevated temperatures of approximately 8-14°C in the red muscle, but not the visceral or cranial regions. CS activity significantly decreased as the PBT grew larger, however, there was a trend for the COX activity to increase in the mid-size muscle and then decrease in larger fish.

**POS-WED-151**

**A DICTYOSTELIUM MODEL FOR ALZHEIMER’S DISEASE AND OTHER TAUOPATHIES**

Mroczek K.M., Fernando S.G., Annesley S.J. and Fisher P.R.

La Trobe University, Vic 3086, Australia.

Alzheimer’s disease is the most prevalent neurodegenerative disorder. It belongs to a group of neurodegenerative diseases known as tauopathies, the hallmark of these being Tau protein aggregates in the brain. Normally, Tau binds dynamically to microtubules, dependent on the protein’s phosphorylation state. In disease-affected neurons, hyperphosphorylation causes the accumulation of Tau protein into aggregates, which colocalise with other disease proteins in neurodegeneration. One such protein is α-synuclein, the main constituent of aggregates in Parkinson’s disease. We have genetically modified the model organism Dictyostelium discoideum to express human Tau alone, and in combination with human α-synuclein. Dictyostelium provides well characterised models for various human diseases, including neurodegeneration. Its cellular processes are comparable to our own and it possesses many homologues of human disease genes. Notably, it does not possess homologues of tau or α-synuclein, providing an opportunity for functional in vivo studies of these proteins singly and in combination, without interference from endogenous homologues. We found that ectopic expression of Tau alone causes a moderate phototaxis defect but has no effect on endocytosis or growth. Expression of α-synuclein alone impaired phototaxis as well as phagocytic uptake of and growth on bacteria. Coexpression of Tau and α-synuclein- mediated phagocytosis/growth defect and produced a more severe defect in phototaxis than either protein alone. This genetic interaction between the two proteins was confirmed by DoudiniaTM immunofluorescence experiments showing that Tau not only interacts with tubulin directly on the microtubules of Dictyostelium, but also with α-synuclein in the cell cortex. D. discoideum thus provides a viable simple model for studying tauopathies and interactions between proteins involved in neurodegeneration.

**POS-WED-150**

**HYPOXIA INDUCES UPREGULATION AND NUCLEAR LOCALIZATION OF DPP4 IN OVARIAN CANCER CELLS**

Moffitt L.R.1,2, Bilandzic M.1,2 and Stephens A.N.1,2

1Hudson Institute of Medical Research, VIC, Australia. 2Monash University, VIC, Australia.

Ovarian cancer accounts for over half of all gynaecological cancer-related deaths in Australian women and has a low 5-year relative survival rate of approximately 40%. A lack of early detection, disease recurrence and the almost universal development of chemoresistance all contribute to this high mortality rate. There is an urgent need to develop targeted therapies to improve clinical outcomes for ovarian cancer patients. Dipeptidyl peptidase 4 (DPP4) is a serine protease that is expressed in many solid tumour types. DPP4 enzymatically regulates the bioactivity of multiple cytokines and immune mediators; it also acts as an adhesion molecule that mediates cell-cell interactions. Within the hypoxic tumour microenvironment, DPP4 is expressed and is present in solid ovarian tumours and tumour-derived spheroids – organoids that promote chemoresistance, disease dissemination and tumour recurrence. Whilst DPP4 is regulated by Hypoxia-Inducible Factor-1α (HIF-1α) in other cell types, the mechanism is cell-specific. The relationship between DPP4 and hypoxia has never been investigated in ovarian cancer. We cultured the OVA/C4R human ovarian cancer cell line in 2% O₂ and analyzed gene expression by quantitative real-time PCR, soluble DPP4 protein levels by ELISA and protein localization by immunofluorescence. We demonstrated upregulation of DPP4 expression and release of soluble DPP4 protein from the cell surface under chronic hypoxia, indicating the potential involvement of DPP4 in the hypoxic response. Additionally, we observed nuclear co-localization of DPP4 and HIF-1 under hypoxic conditions, suggesting a previously undescribed nuclear function of DPP4 and its possible role in the pathogenesis of ovarian cancer. Further investigations into the mechanisms of DPP4 in the ovarian tumour environment may provide a novel insight into improving treatment strategies.

**POS-WED-152**

**COMBATORIAL REGULATION OF UDP-GLUCURONOSYLTRANSFERASE (UGT)-1A8 GENE BY INTESTINAL FACTORS CDX2 (CAUDAL-RELATED HOEeDOMOEAID PROTEIN-2) AND HNF4α (HEPATOCYTE NUCLEAR FACTOR-4 ALPHA)**

Mubarakoh S.N., MacKenzie P.I. and Meech R.

Department of Clinical Pharmacology, Flinders University, Bedford Park, SA, Australia, 5042.

Human intestine is an important first line of defence against dietary toxins and carcinogens: this is largely mediated by UDP-glucuronosyltransferase (UGT) detoxification activities. Intestinal UGT expression starts early during organ formation and is maintained in adults during the dynamic process of intestinal homeostasis/renewal. UGT1A8 is the main UGT isoenzyme specifically expressed in the intestine and is involved in cancer prevention. Low activity of UGT1A8 is associated with elevated risk of oesophageal and colorectal cancer. This study examined the involvement of CdX2 and HNF4α, two key transcriptional controllers of intestinal development and renewal, in regulation of UGT1A8. Using endogenous gene expression and luciferase-reporter assays in Caco-2 cells, we determined that CdX2 and HNF4α synergistically regulate UGT1A8 via a novel HNF4α-consensus motif in the UGT1A8 proximal promoter (nt -44,HNF4α). Binding of HNF4α to the novel element was confirmed by chromatin immunoprecipitation (ChiP) assays. Interestingly, sequence analysis predicted a cryptic CdX2 recognition motif within the -44HNF4α element. Using electrophoretic mobility shift assays (EMSA) we confirmed that the -44HNF4α element binds both HNF4α and CdX2, defining it as a composite element that may mediate the synergistic activation of UGT1A8 by HNF4α and CdX2. Simultaneous knockdown of CdX2 and HNF4α using siRNA decreased UGT1A8 mRNA level indicating that both factors are required for the synergistic activation of UGT1A8 expression. We also demonstrated that CdX2 and HNF4α reciprocally regulate each other creating a feed-forward loop. Preliminary data from intestinal organoids developed from ‘humanized’ UGT1A8-mice showed UGT1A8 expression was comparable to that in adult intestine. This provides scope for future studies to understand how UGT1A8 expression is specified and maintained during intestinal development, homeostasis and regeneration using humanized mice and organoid technology.
Mechanical stresses (stress) are important developmental cues in all organisms. The influence of mechanical stress has largely been characterized in animal and yeast model systems, whereas in plants, the stress sensors and signaling pathways remain largely unknown. Plant cells respond to stress by remodeling their cell walls to maintain integrity during growth. Detection of stress caused by anisotopic cell growth and expansion in actively growing tissues, such as the cotyledon epidermis or shoot apical meristem (SAM), is important for proper wall remodeling and thus formation of organs. We have identified DEFECTIVE KERNEL 1 (DEK1), a plant-specific CALPAIN protease as a putative mechanosensor. Plants with altered levels of DEK1 display growth defects, changes in cell wall structure and composition, predominantly in pectin and cellulose. These phenotypes suggest altered responses to mechanical signals. Using reporter lines for cellulose synthase 6 (CESA6-YFP), our preliminary results show that plants with reduced levels of DEK1 have fewer cellulose synthase complexes (CSCs) in cotyledon pavement cell membranes. These complexes are slower and display more erratic trajectories when compared with wild type plants. Phenotypic analysis of the SAM of a dek1-4 mutant and lines with inducible overexpression of the CALPAIN domain show these lines have defects in floral primordia initiation and sepal development. These data suggest DEK1-regulation of the cell wall impacts SAM development and further investigation of DEK1 will reveal its important roles during plant growth.

Phenotypic analysis of the SAM of a dek1-4 mutant and lines with inducible overexpression of the CALPAIN domain show these lines have defects in floral primordia initiation and sepal development. These data suggest DEK1-regulation of the cell wall impacts SAM development and further investigation of DEK1 will reveal its important roles during plant growth.
ALS-LINKED MUTANT CYCLIN F IMPedes RA81- DEPENDANT ER-GOLGI TRAFFICKING AND INDUCES ER STRESS MEDIATED APOTOTIC CELL DEATH

Ragagnin A.M.G.1, Sundaramoorthy V., Parakh S., Peri E.R., Vidal M., Soo K.Y., Shahheytari H., Chung R., Blair I. and Atkin J.D.A.1,2
1Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, NSW, Australia. 2Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Kingsbury Drive, Bundoora, Melbourne, VIC, Australia.

Intracellular protein aggregates are characteristic of neurodegenerative diseases including amyotrophic lateral sclerosis (ALS), which is characterised by selective death of upper and lower motor neurons. The exact role of protein aggregates in disease pathology is still under debate. Previously, we demonstrated that inhibition of ER-Golgi transport induces ER stress and apoptosis in ALS. Several proteins are linked genetically and pathologically to neurodegeneration in ALS, including SOD1, TDP-43 and FUS. Recently, missense mutations in the CCNF gene, encoding cyclin F, were identified at similar frequencies to those reported in TARDBP and FUS, in cohorts of familial and sporadic ALS cases from diverse geographic populations. However, the pathogenic mechanisms induced by mutant cyclin F remain to be established. Here, we demonstrate that expression of ALS-associated mutant S621G cyclin F leads to its mislocalisation to the cytoplasm. Furthermore, mutant cyclin F inhibits protein transport between the endoplasmic reticulum (ER) and the Golgi apparatus, impairs ER-associated degradation and induces ER stress, leading to apoptotic neuronal death in SH-SY5Y neuronal cells and in mouse primary cortical neurons. The Rab GTPase Rab1 performs multiple roles in ER–Golgi transport. We demonstrate that over-expression of Rab1 prevents induction of apoptosis in cells expressing mutant CCNF. Together, these results provide evidence for ER to Golgi trafficking dysfunction as a pathogenic pathway in cyclin F-mediated disease and that Rab1-mediated ER–Golgi transport may be a novel therapeutic target in ALS.

PROTEIN INVOLVED IN GLAUCOMA

POSTERS

TMCO1: A NOVEL RNA-BINDING NUCLEOLAR PROTEIN INVOLVED IN GLAUCOMA

Sharma S.1, Martin S.1, Wood J.2, Chidlow G.2, Casson R.2 and Craig J.E.1
1Department of Ophthalmology, Flinders University, South Australia, Australia. 2Ophthalmic Research Laboratories, The University of Adelaide, South Australia, Australia.

Glucoma is the leading cause of irreversible blindness in the world. Glucoma comprises a group of optic neuropathies that lead to progressive loss of retinal ganglion cells in the retina in the eye with corresponding visual field loss. Elevated intraocular pressure due to compromised trabecular meshwork function increases the risk of glaucoma. Our group reported genome-wide significant association of single nucleotide polymorphisms (SNPs) in the TMCO1 gene on chromosome 1q24 with blinding glaucoma. SNPs in TMCO1 are also associated with less severe glucoma in populations across the world. Recessive mutations in TMCO1 cause a developmental disorder involving craniofacial and skeletal anomalies and mental retardation. TMCO1 is evolutionarily extremely conserved but its function is unknown. Our work showed that TMCO1 is ubiquitously expressed and encodes a nucleolar protein. The aim of this study was to determine the function of the TMCO1 gene and its role in glucoma pathogenesis. Data show that TMCO1 acts as an RNA-binding protein in the nucleolus and siRNA-mediated knockdown of the gene reduces cell viability. Hence, it has a role in ribosome biogenesis and regulation of cell proliferation. Furthermore, TMCO1 expression is lower in glucoma patients compared to controls, and lower in cultured trabecular meshwork cells from a glucoma patient compared to a normal individual. Progressive reduction in cellularity of the trabecular meshwork has been reported in glucoma; lower TMCO1 expression may contribute to this reduction. Taken together, this study suggests a role of the TMCO1 gene in regulation of cell proliferation, and in compromised trabecular meshwork function in glucoma.

ROLE OF CXCL13 IN HUMAN RETINAL ENDOTHELIAL CELL BIOLOGY: IMPLICATIONS FOR PATHOGENESIS OF DIABETIC RETINOPATHY

College of Medicine & Public Health, Flinders University, Adelaide, Australia.

Diabetic retinopathy frequently complicates diabetes mellitus and is the leading cause of blindness in working-aged adults worldwide. The B cell chemokine, CXCL13, is increased in the eye in mouse diabetic retinopathy. We investigated expression of CXCL13 and receptor, CXCR5, in human retina, and assessed the role of CXCL13 in human retinal endothelial cell (HREC) proliferation and migration, which are processes that characterise retinal neovascularisation in advanced diabetic retinopathy. CXCL13 transcript and protein were detected in human retinal neurons, while CXCR5 transcript and protein were expressed throughout the retina, and by cultured primary HREC isolates (n=5-5 human eye samples). CXCL13 promoter analysis identified transcription factor binding sites associated with molecular pathways - angiogenesis, interleukin and platelet-derived growth factor - that have been linked to diabetic retinopathy. Recombinant human CXCL13 (100 ng/mL) induced migration of an HREC line by 73±9% (student t-test, p < 0.001), and 5 of 7 primary HREC isolates by 84±18% to 135±27% (one-tailed t-test, p < 0.025) relative to control. Antibody neutralization of CXCR5 significantly reduced CXCL13-induced migration. CXCL13 did not induce migration of human umbilical vein endothelial cells, suggesting endothelial heterogeneity in the migratory response. HRECs stimulated with CXCL13 increased phosphorylation of ERK protein. A cell growth assay performed in the presence of CXCL13 failed to show HREC proliferation. These results indicate that CXCL13 is expressed in human retina and induces migration of HRECs through ligation with CXCR5. Our findings suggest a novel role for CXCL13 in HREC-mediated pathology of diabetic retinopathy.
POS-WED-161

DIMINISHED OST3 DEPENDENT N-GLYCOSYLATION OF THE BIP NUCLEOTIDE EXCHANGE FACTOR SIL1 IS AN ADAPTIVE RESPONSE TO REDUCTIVE ER STRESS

Stevens K.L.1,2, Black A.L.1,2, Wells K.M.1,2, Yeo B.K.Y.1,2, Steuart R.F.L.1,2, String J.C.1, Schulz B.L.1,2 and Mousley C.J.1,2

1 Curtin University, School of Biomedical Sciences, Faculty of Health Sciences, Curtin University, Bentley, WA 6102. 2 Curtin University, Curtin Health Innovation Research Institute and Faculty of Health Sciences, Curtin University, Bentley, WA 6102. 3 University of Queensland, School of Chemistry and Molecular Biosciences, Faculty of Science, University of Queensland, Brisbane St Lucia, QLD 4072. 4 Flinders University, Bedford Park, SA 5042.

Kar2 is an essential Hsp70 chaperone and master regulator of endoplasmic reticulum (ER) function. Kar2’s activity is regulated by its intrinsic ATPase activity that can be stimulated by two different nucleotide exchange factors, Sif1 and Lhs1. Both Sif1 and Lhs1 are glycoproteins, however, how N-glycosylation regulates their function is not known. Here we show that N-glycosylation of Sif1, but not of Lhs1, is diminished upon reductive stress. N-glycosylation of Sif1 is predominantly Ost3-dependent and requires a functional Ost3 CxxC thioredoxin motif. N-glycosylation of Lhs1 is largely Ost3 independent and independent of the CxxC motif. Unglycosylated Sif1 is not only functional but can more effectively substitute for Lhs1 activity than N-glycosylated Sif1. We propose that reductive stress in the ER inhibits Ost3 dependent N-glycosylation of Sif1, which regulates specific Kar2 functions appropriate to the needs of the ER under reductive stress.

POS-WED-163

TRAFFICKING AND UPTAKE OF HUMAN SPHINGOMYELIN PHOSPHODIESTERASE ACID-LIKE 3A (SMPDL3A), A NOVEL LXR-REGULATED NUCLEOTIDE PHOSPHODIESTERASE

Traini M.1, Thaysen-Andersen M.2, Loke I.2, Kumaran R.1,2, Kokc D.1, Jessup W.1 and Kristianides L.1

1 ANZAC Research Institute, Sydney Medical School, University of Sydney. 2 Department of Molecular Science, Macquarie University.

Sphingomyelin phosphodiesterase acid-like 3A (SMPDL3A) is a recently identified secreted nucleotide phosphodiesterase. In human macrophages, its expression and secretion is up-regulated by cholesterol loading. Liver X Receptor (LXR) stimulation, and elevated intracellular cAMP. Although SMPDL3A is highly homologous to the well-characterised enzyme acid sphingomyelinase, SMPDL3A cannot hydrolyse sphingosine or phospholipids. Instead, it demonstrates an unexpected preference for nucleotide tri- and diphosphate substrates at acidic pH optimum. Evidence from functional genomics studies suggests the involvement of SMPDL3A in both sterol metabolism and inflammatory signaling, making it a potentially important player in diseases such as atherosclerosis. However, much of its fundamental cellular biology and function remains unknown. To better understand this, we examined aspects of the secretion, intracellular localization and uptake of human SMPDL3A. N-glycosylation of SMPDL3A at specific sites was essential for intracellular stability, secretion and maintenance of enzymatic activity. In human macrophages and HEK cells, intracellular SMPDL3A is delivered to lysosomes. Exogenous recombinant human SMPDL3A is endocytosed and also transported to lysosomes, where it retains enzymatic activity and is highly stable. This uptake is mannose-6-phosphate receptor dependent, and specifically requires N-glycosylation of Asn222 of SMPDL3A. Circulating SMPDL3A from human plasma is not similarly endocytosed by macrophages, suggesting its glycosylation differs from that secreted directly from cells. These results give additional context to the unusual substrate specificity of SMPDL3A and highlight potential functional roles.

POS-WED-162

THE ROLE OF DIMETHYLARGININE DIMETHYLAMINOHYDROLASE 1 (DDAH1) INHIBITION IN EXPERIMENTAL ANGIOGENESIS

Tajbakhsh N.1, Lewis B.C.1, Smith J.R.2, Bonded C.S.2 and Mangoni A.A.1

1 Departments of Clinical Pharmacology, Flinders University, Adelaide. 2 Department of Ophthalmology, Flinders University, Adelaide. 3 Centre for Cancer Biology, University of South Australia, Adelaide.

Introduction. Nitric oxide (NO) interacts with the pro-angiogenic growth factor, vascular endothelial growth factor-A (VEGF-A). Dimethylarginine dimethylaminohydrolase-1 (DDAH1) metabolises the endogenous NO synthase (NOS) inhibitors, asymmetric dimethylarginine (ADMA) and N'G'-monomethylarginine (NMMA). DDAH1 inhibition might halt the excessive angiogenesis in disease states. Aims. To identify dose-response relationships between pharmacological DDAH1 inhibition and angiogenesis. Also to characterise the interaction between DDAH1, NOS, and VEGF-A in a retinal cell model (RPE). Methods. Endothelial tube formation assays were undertaken in HUVEC and Vero/A cells with or without DDAH1 inhibitors. All experiments were conducted over a 24-h period, with tube formation recorded post 5-h incubation in matrigel, mRNA (q-PCR) and protein expression of DDAH1 (western blot) and VEGF-A (ELISA) in RPE were tested to investigate the effect of inhibition on VEGF-A production. Results. Compound 10a (5uM) significantly reduced tube (~40%) and loop (~55%) formation relative to untreated cells. Increasing the concentration of both DDAH1 inhibitors to 10μM reduced the antiangiogenic response (10a: ~25% tube, ~42% loop). Mechanistic studies in RPE revealed VEGF-A expression increase approximately 1.5-fold under hypoxia. The presence of VEGF-A and DDAH1, but not NOS, was confirmed at the mRNA and protein levels. siRNA mediated knockdown of DDAH1 revealed DDAH1 does not directly regulate VEGF-A production in RPE cells deficient in NOS. Discussion. DDAH1 inhibition significantly reduces NO-mediated angiogenesis. Since no direct correlation between DDAH1 inhibition and VEGF-A levels exists in NOS-deficient RPE, future experiments will investigate whether INOS induction mediates VEGF-A production, and whether the mechanism of the observed antiangiogenic response is dependent on the DDAH1/INOS pathway.

POS-WED-164

DETECTION AND CHARACTERIZATION OF A NOVEL EXTRACELLULAR ISOFORM OF FUSED IN SARCOMA (FUS)

Vidal M.1, Lee A.1, Heng B.1, Ragagnin A.1, Sundaramoorthy V.1 and Atkin J.D.1,2

1 Department of Biomedical Sciences, Macquarie University, North Ryde, New South Wales, Australia. 2 Department of Biochemistry and Genetics, La Trobe Institute for Molecular Science, La Trobe University, Bundoora, Victoria, Australia.

The FUS gene encodes a multifunctional protein which is misfolded and mutated in Amyotrophic Lateral Sclerosis (ALS). Mutations in FUS account for 5% of familiar and 1% sporadic ALS cases. Predictions based on a bioinformatics study by Wilson et al. 2014 suggested that two novel isoforms of FUS exist in addition to the canonical well-studied isoform. These new isoforms result from a combination of a frame-shifting splice event and the production of an alternative start codon, changing the reading frame at the amino terminus. Therefore most ALS mutations, clustered at the C terminus, should be present in the novel isoforms. This study aimed to identify and characterize a novel extracellular isoform of FUS and examine its role in ALS. Expression at the mRNA level was confirmed using primers specific for the isoform in human neuronal cell lines and human primary neurons. Mass spectrometry and western blotting revealed the presence of the novel isoform at the protein level in media of SH-SY5Y cells, demonstrating its extracellular location. GFP and HA-tagged constructs encoding the isoform were expressed in SH-SY5Y cells, and western blotting confirmed the extracellular presence of the isoform. Furthermore, deglycosylation with PNGase treatment revealed that it is N-glycosylated. This work is the first to identify the predicted novel extracellular isoform of FUS, which is secreted from human neuron cells, implying a novel physiological function distinct from the canonical isoform. The role of the isoform in ALS is currently being examined.
NEAR-INFRARED FLUORESCENCE AS A NON-INVASIVE METHOD TO MONITOR MURINE OVARIAN TUMOURS IN VIVO

Wilson A.L.1,2, Lovelock T.M.1,2, Bassett B.1,2, Xiang S.2, Bilandzic M.1, Plebanski M.1 and Stephens A.N.1
1Hudson Institute of Medical Research, VIC, Australia. 2Monash University, VIC, Australia.

Epithelial ovarian cancer is the most lethal gynaecological malignancy, despite its relatively low incidence of 12.5 in 100,000 women. Preclinical models that allow the accurate staging of ovarian tumour growth are important in the evaluation of therapeutic responses in vivo. However, there are currently no appropriate, non-invasive approaches to accurately and simply monitor ovarian cancer progression in mice. We have assessed whether tumour cells expressing near-infrared protein (iRFP) allow in vivo tumour imaging, to track disease progression. ID8 mouse ovarian cancer cells were transfected with a vector expressing the bacterial phytochrome iRFP720. ID8-iRFP720 cells displayed near-infrared fluorescence (ex702nm/em720nm), and had identical proliferative and invasive characteristics compared to wild-type ID8s in vitro. Wild-type or ID8-iRFP720 cells were intraperitoneally (iP) implanted into C57BL/6 mice, and tumours imaged over time using an IVIS III In Vivo Imaging System (Perkin Elmer). Tumour growth and dissemination could be tracked according to fluorescence, which increased in ID8-iRFP720 tumour-bearing mice over time until experimental endpoint (week 9). Strong iRFP fluorescence was associated with macroscopic tumour burden. This model will be used to investigate early events in ovarian cancer progression, and to assess new therapeutics in vivo for their translatable potential.

EVIDENCE FOR THE INTERACTION OF PEROXIREDOXIN-4 WITH THE STORE-OPERATED CALCIUM CHANNEL ACTIVATOR STIM1 IN LIVER CELLS

Tam K.C., Ali E., Hua J., Chataway T.K. and Barratt G.J.
Flinders University, Adelaide, South Australia, 5001.

Ca\(^{2+}\) entry through store-operated Ca\(^{2+}\) channels (SOCs) in the plasma membrane (PM) of hepatocytes plays a central role in the hormonal regulation of liver metabolism. SOCs are composed of Orai1, the channel pore protein, and STIM1, the activator, protein, and are regulated by hormones and reactive oxygen species (ROS). Most studies to date have employed immortalised cultured cells transfected with GFP- or YFP-tagged Orai1 and STIM1. Little is known about the intracellular distribution of endogenous Orai1 and STIM1. Moreover, STIM1 may interact with several other intracellular proteins. The aims are to determine the intracellular distribution of endogenous Orai1 and STIM1 in hepatocytes and to identify novel STIM1 binding proteins. Subcellular fractions of rat liver were prepared by homogenisation and differential centrifugation. Orai1 and STIM1 were identified and quantified by western blot. Orai1 was found in the PM (0.03%), heavy (44%) and light (53%) microsomal fractions, and STIM1 in the PM (0.09%), heavy (85%) and light (13%) microsomal fractions. Immunoprecipitation of STIM1 followed by LC/MS identified peroxiredoxin-4 (Prx-4) as a potential STIM1 binding protein. This was confirmed by co-immunoprecipitation experiments. Prx-4 was found principally in the heavy microsomal fraction. Knockdown of Prx-4 using siRNA, or inhibition of Prx-4 using conodin A, did not affect Ca\(^{2+}\) entry through SOCs but enhanced the inhibition of SOCs by hydrogen peroxide. It is concluded that (i) a considerable amount of Orai1 is located in intracellular membranes and (ii) STIM1 interacts with Prx-4, which contributes to the regulation of STIM1 and SOCs by ROS.

THE FGF SIGNALLING PATHWAY IN CORALS: EVOLUTION AND POTENTIAL ROLE DURING DEVELOPMENT

Fortunato S.A.V., Moya A. and Miller D.J.
ARC Centre of Excellence for Coral Reef Studies, James Cook University.

Coral transcriptomic data is quite extensive, but despite this, little is known about signalling pathways involved in developmental traits and stress responses. Corals are under threat, therefore understanding these pathways are crucial not only for expanding knowledge in developmental biology and evolution, but also for efficient coral reef management. The present study aimed to analyze the evolution and potential role of the Fibroblast growth factor signalling pathway in corals. The FGF signalling pathway has a key role during development in animals, from cnidarians to mammals. In Cnidaria, thirteen FGF ligands and three FGF receptors have been described in anemones and hydrozoans. Few ligands are ortholog of the Bilateria FGF 8/17/18 family, while the remaining do not belong to any known family. In the anemone Nematostella vectensis, one of the current models for developmental stuctures in Cnidaria, the FGF pathway has key roles throughout development. To identify the homology of FGF ligands and FGFR in corals, phylogenetic analyses were performed using an expanded dataset of genomic data in Cnidaria, including two of our recently sequenced coral transcriptomes. The phylogenetic analyses suggest that there is an extended repertoire of FGF ligands in Cnidaria, with novel families not previously described, which are specific to either Cnidaria or to corals. In addition, spatial- temporal expression analyses of FGF ligands and receptors were performed in corals. Our findings suggests a potential role of the FGF signalling pathway during skeletal development in corals.

INVESTIGATING THE CELLULAR DISTRIBUTION OF 5-HT3 RECEPTOR SUBUNITS USING TIRF MICROSCOPY AND DOT ANALYSIS

Abad I.P.L., Nowell C., Fam R., Trinh P., Yaakob N., Manallack D. and Irving H.
Monash Institute of Pharmaceutical Sciences, Monash University, Parkville Campus, VIC, 3052.

The 5-hydroxytryptamine (5-HT) type 3 (5-HT\(_3\)) receptor is expressed highly in the G1-tract and in various regions of the brain and the blockade of the 5-HT\(_3\) receptor is clinically used as an antiepileptic treatment of chemotherapeutic-induced nausea. All 5-HT receptors are G-protein coupled receptors except the 5-HT\(_3\) receptor which is an ion channel known to form functional cation-permeable pores by expressing either homomers or heteromers of its five possible 5-HT\(_3\) subunits (A-E). Although the 5-HT\(_3\)A subunit is known to be crucial to the formation of the pore, in vitro, stoichiometry, pharmacology and function of the 5-HT\(_3\)A heteromer is yet to be studied in detail. To investigate the co-expression of the subunits, human 5-HT\(_3\)- and SH5 plasmids were each fused with GFP and mCherry in the second intracellular loop, transformed into pDest40 vector, transfected into HEK293T in varying 5-HT\(_3\)A to 5-HT\(_3\)A (A:C) ratios and were imaged using confocal and TIRF microscopy. A protocol was developed to optimize the A:C ratio to 1:9 accommodating the observed lower signal of the 5-HT\(_3\)A compared to its 5-HT\(_3\)A counterpart. This could be due to a lower expression rate or inefficient protein folding of the 5-HT\(_3\)A subunit. Dot analysis of the TIRF imaging suggested that both 5-HT\(_3\)A and 5-HT\(_3\)B subunits could be successfully co-expressed at the cell membrane. We report on the ratio of subunits colocalized at the plasma membrane and within the cells and how this is influenced by exposure to the ligand 5-HT. This method of investigating the 5-HT\(_3\) receptor could influence the drug design for 5-HT\(_3\) receptor related targets with the goal for higher selectivity pending further probing into the stoichiometry and assembly of these receptor subunits.
POS-WED-169

INSIGHTS INTO MODULATION OF NFKB FUNCTION BY IRAK3

Freihat L.A., Manallack D.T., Wheeler J.I. and Irving H.R.
Drug Discovery Biology, Monash Institute of Pharmaceutical Sciences - Monash University.

Interleukin-1 receptor associated kinase 3 (IRAK3) is a negative regulator of innate immune signaling. IRAK3 is a proposed diagnostic and prognostic marker in inflammation, and possibly a target for intervention in several inflammatory related diseases. The exact mechanism of action and the selectivity of IRAK3 is however still largely unclear and further evaluation is needed. Prior studies identified IRAK3 as a potential novel guanylate cyclase (GC) catalyzing cyclic guanosine monophosphate (cGMP) synthesis. Using HEK BLUE NLIR6 cells, we have confirmed that wildtype IRAK3 functions to reduce lipopolysaccharide stimulated NFκB activity. However, point mutant GC activity deficient IRAK3 did not reduce NFκB activity in these cells. The negligible effect of the IRAK3 mutants can be reversed in the presence of added external membrane permeable 8-Br cGMP. The cGMP produced by IRAK3 may be involved in its regulatory function, where cGMP could affect selectivity in downstream signaling pathway(s). cGMP may function to modulate binding and/or activity of nearby interacting proteins involved in the cascade. Our findings may provide insight into the mechanisms underpinning the inhibitory actions of IRAK3 in the inflammatory signaling cascade.

POS-WED-171

DOES PI3K/AKT SIGNALLING DRIVE THE WARBURG EFFECT IN THE MAMMALIAN RETINA?

Haydinger C.D., Casson R.J. and Peet D.J.
1School of Biological Sciences, University of Adelaide, Adelaide, Australia. 2South Australian Institute of Ophthalmology, University of Adelaide; and Hanson Institute, Adelaide, Australia.

The mammalian retina converts a large proportion of glucose to lactate even when oxygen is available. This is known as aerobic glycolysis or the Warburg effect, and is common in proliferating cells, especially cancers. Research has identified several signalling pathways that contribute to driving the Warburg effect in cancer, but it is not known whether the same pathways drive the effect in the retina. PI3K/Akt signalling is one such pathway, which amongst diverse cellular effects can upregulate glycolytic enzymes and glucose transporters in response to growth factors. Its unregulated activation has been shown to drive lactate production in cancer. Here, we investigate the role of the PI3K/Akt pathway in glucose metabolism in the mammalian retina. Preliminary experiments measured the effect of chemical inhibition of PI3K and Akt on lactate production in cultured rat retinal Muller glial cells, and we are extending this work to rat retinal explants. Concurrently, we are analysing differential expression of metabolically important genes among retinal cell types to identify metabolic differences and cell populations that likely exhibit the Warburg effect. Understanding the energy demands of the retina may provide a basis for development of therapeutic strategies to treat retinal ischemic diseases, in which a deficit of metabolic substrates contributes to disease pathology. Moreover, if the pathways that drive the Warburg effect in the retina are the same as in cancer, then cancer therapies targeting those pathways may have adverse effects on the retina.

POS-WED-170

DECIPHERING THE LAMININ-511 -YAP/TAZ-ER SIGNALLING AXIS IN TRIPLE NEGATIVE BREAST CANCER

Fuentes M.1, 2 and Pouliot N.1, 2
1Matrix Microenvironment & Metastasis Laboratory, Olivia Newton John Cancer Research, Heidelberg, VIC, Australia. 2School of Cancer Medicine, La Trobe University, Bundoora, VIC, Australia.

Treatment of metastatic breast cancer remains a significant challenge for physicians. In particular, Triple Negative Breast Cancers (TNBCs) that lack oestrogen (ER), progesterone (PR) and HER receptors are resistant to anti-oestrogen or HER2-targeted therapy. Accordingly, new therapies are needed for metastatic TNBC which accounts for 15-20% of tumours and is associated with poor survival. Laminin-511 (LM-511) is a basement membrane protein whose high expression and role in TNBC progression is well established. Recently, we found that interaction of TNBC cells with Lam-LM-511 regulates epithelial plasticity (EMT/MET) and that these phenotypic changes are associated with altered subcellular localisation of the Hippo transducers YAP/TAZ and ER expression. Here, we aim to characterise the interplay between LM-511 receptors, YAP/TAZ/EMT effectors and ER expression. We hypothesise that LM-511 and its integrin receptors regulate ER expression and function in TNBC via a YAP/TAZ-EMT effectors signalling axis. Using a gene knockdown approach, we show that suppression of LM-511 results in loss of TAZ nuclear localisation and de novo ER expression. These observations raise the exciting possibility that ER expression and response to anti-oestrogens could be restored in patients with metastatic TNBC by inhibiting LM-511/integrin signalling. Experiments are underway to confirm whether knockdown of integrin receptors, YAP, TAZ or selected EMT effectors can mimic the effect of LM-511 suppression and induce ER expression and function in human TNBC lines. 1. Boyle, P. (2012). Triple-negative breast cancer: epidemiological considerations and recommendations. Annals of oncology, 23(suppl_6), vi7-vi12. 2. Pouliot, N., & Kusuma, N. (2013). Laminin-511: a multi-functional adhesion protein regulating cell migration, tumor invasion and metastasis. Cell adhesion & migration, 7(1), 142-149.

POS-WED-172

DOES THE GLYCOLYTIC ENZYME PKM2 PLAY A ROLE?

Kittipassorn T.1, 2, Wood J.P.1, 3, Mammone T.1, 3, Casson R.J.1, 3 and Peet D.J.1
1University of Adelaide, Australia. 2Mahidol University, Thailand. 3Hanson Institute, Australia.

Surprisingly similar to most cancer cells, light-sensing photoreceptors and Müller glial cells of the mammalian retina display the Warburg effect (aerobic glycolysis), an unusual metabolism whereby the cells tend to convert glucose into lactate via glycolysis regardless of oxygen availability. In cancer, the glycolytic enzyme Pyruvate Kinase M2 (PKM2) promotes lactate production and acts as a coactivator for the transcription factor Hypoxia-Inducible Factor-1 (HIF-1), stimulating glycolysis; thus, PKM2 is implicated in driving the Warburg effect. We hypothesize that PKM2 also drives the Warburg effect in the retina. Here we demonstrate that photoreceptors express PKM2 and provide the first evidence of PKM2 expression in primary Müller cells and the immortalised Müller cell line rMC-1. We characterise metabolic profiles of these Müller cells, and confirm that they display the Warburg effect. PKM2 knockdown does not significantly affect glucose metabolism in rMC-1 cells, consistent with PKM2 not driving the Warburg effect in Müller cells. However, PKM2 knockdown leads to a decrease in cell number, suggesting a role for PKM2 in Müller cell proliferation and/or survival. To further investigate this, we are generating PKM2 knockout rMC-1 cells and a photoreceptor-specific PKM2 knockout mouse, using CRISPR-CAS9 technology. We have also generated a novel Spontaneously Immortalized Rat Müller cell line (SIRMu-1), which is a useful tool to study the retina. Our research will help design improved treatments for retinal diseases associated with abnormal retinal metabolism. Additionally, as PKM2 may play a role in Müller cell survival, novel cancer treatments targeting PKM2 might damage the retina.
**POSTERS**

**POS-WED-173**

**DUAL SPHINGOSINE KINASE AND BCL-2 INHIBITION EXHIBITS SYNERGISTIC CELL DEATH IN ACUTE MYELOID LEUKEMIA**

Lewis A.C.1,2, Powell J.A.1 and Pitson S.M.1

1Centre for Cancer Biology, University of South Australia and SA Pathology, Frome Road, Adelaide, SA 5000, Australia. 2University of South Australia, School of Pharmacy and Medical Sciences, Adelaide, SA 5000, Australia.

Pro-survival Bcl-2 family proteins such as Mcl-1 and Bcl-2 have garnered significant interest as therapeutic targets due to their up-regulation in many cancers, including acute myeloid leukaemia (AML), leading to enhanced cancer cell survival. Small molecule inhibitors such as the selective Bcl-2 inhibitor, Venetoclax, are very effective in some cases, but have demonstrated poor single agent efficacy in AML due to these cells being highly dependent on Mcl-1, which is not targeted by this agent. Sphingosine kinase 1 (SK1) is a signalling enzyme with established roles in oncogenesis and has recently emerged as a potential therapeutic target in leukaemia. We recently demonstrated that the selective SK1 inhibitor, MP-A08 exhibits anti-leukemic activity in vitro and in vivo using patient derived AML xenograft models. MP-A08-mediated cytotoxicity in AML cells correlated with a reduction in Mcl-1 levels, as well as upregulation of B3H only proteins. Here, we found that combinatorial therapies with MP-A08 and Venetoclax induced synergistic cell death in AML cell lines and patient samples. Mechanistically, MP-A08 induces transcriptional upregulation of BH3 only protein, Noxa and formation of Noxa/Mcl-1 complexes. MP-A08 appears to exert its cytotoxicity in AML cells through loss of Mcl-1 as a consequence of Noxa binding. This data provides impetus to investigate dual MP-A08 and Venetoclax administration in vivo to validate as a potential therapeutic strategy in AML.

**POS-WED-175**

**NOVEL PATHWAYS TO REGULATE LEVELS OF STEROID HORMONES AND STEROID SIGNALING IN CANCER**

Meech R.1,2, Hu D.-G.1, Chanawong A.1, Hulin J.-A.1, McKinnon R.A.2 and Mackenzie P.I.1

1Flinders University. 2Flinders Centre for Innovation in Cancer.

Breast and prostate cancer growth is primarily regulated by steroid signaling via androgen and estrogen receptors (ER/AR). Loss of receptor expression or their overexpression may be exploited to selectively target androgen or estrogen-dependent states that are generally more aggressive and that preclude treatment with hormonal therapies. While much is known about the regulation of ER and AR expression and function, less is known about how steroid levels are regulated. Several members of the UDP Glucuronosyltransferase (UGT) family conjugate steroid hormones with sugar groups: this is the only known irreversible reaction that renders steroids inactive as ligands. The UGT isoforms UGT2B15 and UGT2B17 conjugate natural androgens as well as steroidal therapies such as SERMs and aromatase inhibitors. These UGTs are of particular interest in breast and prostate cancer where their expression correlates with survival outcomes, presumably through regulating levels of natural steroids as well as steroidal drugs. We have also shown that both UGT2B15 and UGT2B17 activities are potentiated by both estrogens and androgens via direct binding of ER and AR to their promoters. This suggests a tight feedback loop through which these receptors can control the levels of their own ligands in cancer cells. We have investigated the requirement for UGT2B15 and UGT2B17 activities in steroid dependent signaling by deleting these genes from the ER+AR+ breast cancer cell line MCF7 using CRISPR. UGT2B15 and UGT2B17 knockout cell lines show altered cell morphologies, proliferation rates and invasion capacities relative to wildtype cells, consistent with altered steroid-dependent gene regulation.

We have also characterized novel splice variants of these UGTs that can cause different signaling responses and patterns in Drosophila melanogaster cells. We also found that one novel variant showed high toxicity to Helicoverpa armigera (cotton bollworm) without affecting D melanogaster flies and cells. We propose that combining these physiological and cellular techniques is likely to assist the discovery of new biopesticides, and the identification of new modes of action for insecticidal activity.

**POS-WED-176**

**ROLE OF THE QUAKING ISOFORMS IN ALTERNATIVE SPlicing DURING PROSTATE CANCER EPITHELIAL-MESENCHYMAL TRANSITION**

Neumann D.1, Phillips C.A.1, Touba J.1, Pillman K.1, Roslan S.1, Seth L.A.1, Hollier B.G.2, Goodall G.J.1,3 and Gregory P.A.1,3

1Centre for Cancer Biology, University of South Australia and SA Pathology, Adelaide, SA 5000, Australia. 2Institute of Health and Biomedical Innovation, Australian Prostate Cancer Research Centre - Queensland, Princess Alexandra Hospital, Queensland University of Technology, Queensland, Australia. 3Discipline of Medicine, The University of Adelaide, Adelaide, SA 5005, Australia.

Metastasis is the major cause of death in epithelial-cell derived cancers, including prostate cancer. Epithelial-mesenchymal transition (EMT) is an important step in metastasis that involves morphological and functional characteristics which contribute disease progression and is driven by key transcription factors including members of the Snail and ZEB families. Using Snail1 and ZEB1 inducible models of prostate cancer EMT, we found that several isoforms of the RNA binding protein QKI are strongly upregulated during EMT concomitant with changes in alternative splicing of several genes including ADD3, NUMB and CD47. By knockdown of individual QKI isoforms we identified that the QKI-5 isoform, and not QKI-6 and QKI-7, are likely to drive these changes. Further investigation of the roles of individual QKI isoforms in prostate cancer progression are ongoing. These data indicate that QKI acts as a major contributor to alternative splicing events regulated during prostate cancer EMT.

**POS-WED-177**

**EXPLORING MODES OF ACTION OF NOVEL BIOPESTICIDES: FROM CELL LINE TO TARGET INSECTs**

Mak M., Basta A., Beattie K.D., Randall D., Spooner-Hart R.N. and Chen Z.

School of Science and Health, Western Sydney University, Locked Bag 1797, Penrith, NSW 2751, Australia.

An insecticidal challenge lies before the agricultural community. The adoption of genetically modified crops has seen a welcome reduction in insecticide applications, although it has been accompanied by increasing development of resistance by targeted insects in the field. The reduced use of broad-spectrum insecticides also creates a niche opportunity for secondary pests to become more important. This investigation looks at the effects of novel and known biopesticides on insect cell lines and target arthropod species using an array of assays. *Drosophila melanogaster* cell growth assay, electrophysiology, confocal microscopy, and whole insect bioassay have enabled comparisons between fractions of selected novel plant extracts. Cell assays and insect bioassays have identified three novel extracts with insecticidal potential, with high cell inhibition and insect mortality at low concentrations. Electrophysiology results indicated at least one of these extracts has dual activity involving modulation of both potassium and sodium ion channels, while a second extract may suppress ion movement, and the third extract may affect cell mortality via a non-neuronal mode-of-action. Moreover, confocal imaging of H$_2$O$_2$ (a key stress-inducible secondary messenger) showed that the extracts cause differential signaling responses and patterns in *Drosophila melanogaster* cells. We also found that one novel extract showed high toxicity to *Helicoverpa armigera* (cotton bollworm) without affecting *D melanogaster* flies and cells. We propose that combining these physiological and cellular techniques is likely to assist the discovery of new biopesticides, and the identification of new modes of action for insecticidal activity.
POSTERS

POS-WED-177

EFFECT OF DIFFERENT GROWTH FACTORS ON MELANOMA SIGNALLING PATHWAYS

Chan X.Y.1, Singh A.1, Osman N.1, Boyle G.M.2 and Piva T.J.2
1School of Health & Biomedical Sciences, RMIT University, Bundoora, VIC 3083, Australia. 2QIMR Berghofer Medical Research Institute, Herston, QLD 4006, Australia.

Vemurafenib (PLX4032) is often used to treat melanomas that possess the BRAFV600E mutation. While the tumours are shown initially to regress, they become resistant to the drug and the patient relapsed and eventually dies. It has been shown that the growth factors secreted by adjacent cells activate signalling pathways in these melanoma cells. Using specific signalling pathway inhibitors, we investigated the growth factor activation of intracellular signalling pathways in four melanoma cell lines. MM418-C1 (1° tumour) and D24 (2° tumour) cells possess the BRAFV600E mutation, while MM329 (1° tumour) and D24 (2° tumour) cells do not. Growth factors (HGF or TGFα) were added to cells that had been grown for 24 h in low serum (0.5% FBS) containing media, and the expression of p-BRAF, p-Akt, p-ERK1/2, p-PI3K cycling, p- JNK1/2 were quantified using Western blots. From preliminary data we observed that HGF signalled through BRAFV600E in MM418-C1 cells, but TGFα signalled through BRAFV600E in D24 cells. However, expression of other downstream signalling intermediates (p-ERK1/2, p-p38 and p-JNK1/2) of BRAF was increased when either HGF or TGFα was added to the cell lines. We also observed the activation of pAkt (Ser473 and Thr380) by both growth factors which suggests that the PI3K-AKT-mTOR pathway is active in these cell lines. The effect of these growth factors and/or specific signalling pathway inhibitors on these pathways were examined and the significance of the results will be discussed.

POS-WED-179

COMPARATIVE METABOLOMICS IN EUCALYPT ROOT TIPS REVEAL RESPONSE PATTERNS SPECIFIC TO LIFESTYLES OF MICROBES DURING EARLY STAGE OF INTERACTION

Wong J.1, Jayasinghe N.2, Netera S.2, Roessner U.2, Anderson I.1 and Plett J.1
1Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2750, Australia. 2Metabolomics Australia, The University of Melbourne, Parkville, VIC 3010, Australia.

Eucalypts have significant ecological and economical values in Australia. The sustainability of eucalypt forests is heavily influenced by the interactions between soil microbes and the eucalypt root system. Mutualistic microbes enhance nutrient uptake and stress tolerance of their hosts while pathogens weaken or kill eucalypts. Currently we do not have a mechanistic understanding of how Eucalyptus roots are able to differentiate and respond to the broad range of microbes that inhabit forest soils. Using Eucalyptus grandis as a model organism, we sought to characterise the metabolic changes induced in eucalypt roots by soil-borne microbes displaying different lifestyle strategies: Piptopirus microcarpus (mutualistic ectomycorrhizal fungi), Sutulus granulosus (incompatible ectomycorrhizal fungi) and Armillaria luteobubalin (root rot pathogen), as well as one oomycete species Phytophthora cinnamomi (root rot pathogen). By adopting an untargeted metabolite profiling approach, we have been able to identify hundreds of metabolites actively produced in roots during the earliest stages of interaction with these different microbes. We have also identified several eucalypt metabolite response patterns that are specific to a particular microbe and have identified several putative metabolite biomarkers that indicate early onset of pathogenic infection vs. mutualistic colonisation. Our research outcome highlighted the ability of plants to differentiate between different microbes. Specialized metabolites could be produced by plants in the early stage of recognition as a defensive mechanism towards pathogens. The implication of these results will be discussed.

POS-WED-180

USING THE JIGSAW TECHNIQUE TO TEACH ANATOMY

Oakes D.J., Hegedus E.H., Oilerenshaw S.L. and Ritchie H.E. School of Medical Sciences, University of Sydney.

The Challenge: To develop effective active learning experiences that benefit/complement traditional Anatomy laboratory teaching whilst engaging and challenging the students and maximize the benefits of student learning within a small group environment (1). Introduction: This study tested an instruction method that incorporates active-learning in the context of both small groups and peer instruction (1). The model was based on the jigsaw method first described by (2). We present data on the application of the jigsaw technique in an undergraduate Health Sciences setting. Method: First year Medical Radiation Science students participated in a jigsaw workshop on Abdominal Anatomy. Students initially became an expert on one of four parts of the topic and subsequently taught their topic to other students in their working (jigsaw) group. Students completed a pre- and post-workshop quiz to assess their knowledge of Abdominal anatomy and an evaluation survey which is they were asked to rate their educational experience of the jigsaw workshop. The overall Unit of Study performance of Jigsaw participants was compared to those who chose not to attend (non-participants). Results: Students rated the jigsaw session highly for educational value and enjoyment. Quiz scores improved from the pre- to post-jigsaw quiz, showing knowledge gain of Abdominal Anatomy. This occurred even with students who had not yet received formal lectures or practical class relating to the topic. There was a trend in overall improved performance in the Unit of Study of workshop participants compared to non-participants but this improvement was not significant. Conclusions: The jigsaw method provides a useful model to develop peer teaching skills amongst undergraduate students. 1. Sugand K et al (2010) Anatomical Sciences Education, 3(2), 83-93. 2. Aronson E & Bridgeman D (1979). Personality and Social Psychology Bulletin, 5(4), 438-446.
POSTERS

POS-WED-181

INFLUENCE OF LEARNING READINESS AND ATTITUDE TOWARD SOCIAL MEDIA ON LEARNING OUTCOMES IN A PRE-DENTAL FLIPPED CLASSROOM

Roh S. and Ihm J.
Seoul National University School of Dentistry.

A flipped classroom is an instructional strategy that has been introduced recently; however, it is still debated as to how well it works. This study investigates whether pre-dental students’ learning readiness (LR) and attitude toward social media (ASM) empirically influences their learning outcomes in a flipped classroom. Out of 82 pre-dental students in Seoul National University School of Dentistry who signed up for ‘Biodiversity and Global Environment’ class, a 15-week 3-credit course, 61 (74.3%) answered the paper-based survey. The course was designed on the basis of flipped learning where students were supposed to view course materials (video lectures) online before the class session, take the scored written test, and join the group discussion or debate in class facilitated by tutors. At the end of the course, they were asked to rate their LR and ASM using a five-point Likert survey (Gunawardana & Dumphorne, 2001; Mostafa, 2015). Pearson correlations were calculated for LR, ASM, and performance scores, such as written test and tutor evaluation. The high Cronbach’s scores indicated that there is consistency and reproducibility of the LR (Alpha = 0.901) and ASM (Alpha = 0.723) measures. Most students felt a little more prepared before going to class (mean score 3.17 on a 5.0 scale) and expressed a relatively low preference for social media use (mean score 2.49). Thus, it was found that LR in a flipped classroom was significantly correlated with written test (r = 0.361, p = 0.042) and tutor evaluation (r = 0.371, p = 0.035). However, ASM was not significant and negatively correlated with performance scores. This study offered insights into designing a flipped learning course in terms of pre-dental students’ LR and their ASM. Success of flipped learning depends on students’ self-perceived level of preparedness, and much still remains to be done to apply social media benefits in a flipped learning context.

POS-WED-182

DEMONSTRATOR DEVELOPMENT: A ROLE FOR EFFECTIVE QUESTIONING TECHNIQUES FOR TERTIARY EDUCATION PRACTICAL ACTIVITIES

Hu W., Willems-Jones A.1 and Osman A.2
1Department of Biochemistry and Molecular Biology, Medical Building 181, The University of Melbourne 3010. 2Melbourne Graduate School of Education, 234 Queensberry Street, The University of Melbourne 3010.

Laboratory demonstrators are pivotal to successful teaching of science at the undergraduate level. Despite their multifaceted role, the educational background of a demonstrator is often that of laboratory research with no formal teaching training and minimal pedagogical understanding. This study explored the understanding and use of questioning techniques of eighteen laboratory demonstrators, in a second year practical-based subject in The University of Melbourne, before and after a targeted professional development workshop. The suite of questioning techniques included in the workshop was drawn from evidence supplied from research and literature studies. Data analysis of an initial self-evaluation survey, feedback from the workshop, and follow-up observations of the participants in their practical classes showed an improved appreciation and pedagogical understanding of the role of questioning in teaching and learning following the targeted workshop. We also observed that participants displayed real concern for and interest in the quality of the learning experiences they provided to their students in their role as a demonstrator. We believe that it is both feasible and fruitful to develop and evaluate a professional development opportunities for casual teaching staff involved in tertiary science education. The present study contributes to the literature by suggesting important directions for enhancing practical class demonstrators teaching capacity, through a short, yet effective targeted professional development workshop.

POS-WED-183

ROCK SIGNALLLING AND TISSUE STIFFNESS IN EPIDERMAL HOMEOSTASIS

Kular J.1, Scheer K.G.1, Pyne N.T.1, Moretti P.1, Cowin A.J.2, Woodcock J.M.1, Pelton S.M.1,2, Ramshaw H.S.1,2, Lopez A.F.1,3 and Samuel M.S.1,3
1Centre for Cancer Biology, University of South Australia, Adelaide, South Australia. 2Future Industries Institute, University of South Australia, Mawson Lakes 5095, Australia. 3Faculty of Health Sciences, School of Medicine, University of Adelaide, Adelaide 5000, Australia.

The importance of mechanical force in the regulation of skin homeostasis is becoming increasingly appreciated. However, the molecular mechanisms underlying the interplay between force and cell signalling remain unclear. The Rho-ROCK signalling pathway lies at the interface between mechanical and biochemical signalling. We have previously shown that Rho-ROCK signalling promotes keratinocyte proliferation by increasing ECM production, elevating dermal stiffness and enhancing integrin-mediated mechanotransduction signalling. In turn, elevated dermal stiffness further stimulates ROCK activation, setting up a positive feedback loop that, if left unrestrained, promotes cutaneous tumours. We have identified a negative feedback mechanism that limits excessive ROCK signalling in the skin during wound healing but that is lost in squamous cell carcinomas. Signalling through ROCK is selectively tuned down by the molecular adaptor protein 14-3-3zeta, which interacts with an antagonist of ROCK signalling, Mypt1, to maintain it in its active state. The factor(s) regulating the interaction between 14-3-3zeta and Mypt1 in epidermal homeostasis are currently unknown. Here, we show that LKB1/Naun1 signalling regulates MLC activation and phosphorylation by modulating the interaction between Mypt1 and 14-3-3zeta. Our results reveal a novel mechanism that negatively regulates mechanoo-reciprocity and regulates epidermal regeneration, suggesting new therapeutic opportunities.

POS-WED-184

UBIQUITINATION REGULATES CASPASE-2 ACTIVATION AND FUNCTION

Lim Y.1, Bellis D.D.1, Sandow J.2, Dorstyn L.1 and Kumar S.1
1Centre for Cancer Biology, University of South Australia, Adelaide, SA 5001, Australia. 2The Walter and Eliza Hall Institute of Medical Research, Parkville, VIC 3052, Australia.

Anepidermolysis and chromosomal instability are hallmarks of cancer, predominantly caused by mitotic errors. Loss of caspase-2, enhances tumour development in various mouse models (EμMyhc, MMTV-c-neu, Atm), moreover Casp2-/- mouse tumours exhibit increased aneuploidy, that is likely linked to its tumour suppressor function. Our recent and other studies have demonstrated that caspase-2 has a role as tumour suppressor by preventing aneuploidy through either apoptotic deletion of mitotically aberrant and aneuploid cells or cell cycle arrest following increased number of centrosomes and cytokinesis failure. Importantly, the activation of caspase-2 is a key essential mediator of both pathways. However, it is unknown how caspase-2 activation is regulated. To better understand the regulation of caspase-2 activation and activity, we performed proteomic analysis of caspase-2 and identified several novel post-translational modifications of caspase-2 including ubiquitination and phosphorylation. Indeed, for the first time, we showed that caspase-2 is ubiquitinated. Mutation of these ubiquitination sites identified a few which reduced caspase-2 auto-cleavage and apoptotic activity compared to WT caspase-2 when expressed in U2OS- CASP2-/- cells and Casp2-/- MEFs. Further protein stability analysis showed that one mutant is most stable and the GST recombinant protein lost its enzymatic activity in a caspase activity assay. Our data suggest that ubiquitination of caspase-2 may be biologically important in regulating its activation and stability. Further studies will demonstrate the importance of ubiquitination in caspase-2 activation and function in preventing aneuploidy and tumourigenesis.
GENOMIC INSTABILITY IN ADVANCED CHRONIC MYELOID LEUKAEMIA IS CAUSED BY RAG MEDIATED TRANPOSITION

Thomson D., Schreiber A. and Branford S. Centre for Cancer Biology, SA Pathology.

The recombination-activation genes (RAG1 and RAG2) play an essential role in acquired immunity in developing lymphocytes by processing the antigen receptor locus to form a diverse array of immunoglobulins or T-cell receptors. Recently, it has been shown that trans RAG activity is associated with chromosomal translocations and deletions in lymphoid malignancies. We reveal that transposition by RAG1 and associated genes, promotes genomic instability in advanced stages of chronic myeloid leukaemia (CML), with the detection of mobilised DNA at RAG1 target sites. RNAseq and whole exome sequencing analysis of a CML patient cohort (n=144) provide evidence of breakpoints and fusion transcripts in a pattern mimicking V(D)J recombination mediated by RAG1 but in widespread genomic loci. Patients with the poorest prognosis show greater than 1000-fold increase in expression of RAG1, RAG2 and the associated nucleotidyltransferase DNTT at lymphoid blast crisis in comparison to diagnosis, with an accumulation of breakpoints and fusion transcripts at genes associated with lymphoid tissue structure and development. Unbiased motif enrichment searches reveal that breakpoints and associated fusion transcripts are enriched at loci with the recombination signal sequence (RSS) motifs targeted by RAG1. We make the observation that the RSS motif is a component of the repetitive AluSz SINE element [Fig 1d], explaining the pervasive RAG1 targeting and alluding to the previously observed evolutionary link that RAG1 is a domesticated transposon. We propose a mechanism by which transcripts highly expressed in developing lymphocytes, and prone to double-stranded DNA breaks, become the template for transposition at RAG1 mediated double stranded breaks, including known and novel dysregulated genes in CML such as MALAT1, PRKCB, BCR, RUNX1, FOXP1 and ZEB2. Disease progression of CML and resistance to treatment by tyrosine kinase inhibitors (TKIs) is associated with accumulation of genomic lesions, this work provides evidence that transposition by RAG1 is a major driving force behind genomic instability in advanced CML.

CELL SURFACE CONTROL OF MHC II BY MARCH1 E3 UBIQUITIN LIGASE

Liu H.1, Webb A.I.2, Villadangos J.A.1, 3 and Mintern J.D.1
1Department of Biochemistry and Molecular Biology, The University of Melbourne, Parkville, VIC, Australia. 2Systems Biology and Personalised Medicine Division, The Walter and Eliza Hall Institute of Medical Research, Parkville, Australia. 3Department of Microbiology and Immunology, The University of Melbourne, Parkville, VIC, Australia.

Membrane associated RING-CH (MARCH) 1 is a RING type E3 ligase that controls the cell surface abundance of key immunoreceptors such as MHC II and CD86 by ubiquitination. Although previous studies have addressed the physiological functions of MARCH1, not much is known about the mechanisms of its activity. Here, we have undertaken several studies to characterise the ubiquitination of MHC II by MARCH1. First, we quantified the MHC II-associated ubiquitin chain linkages present in primary murine immune cells by in-vitro deubiquitination assay and AQUA mass spectrometry. We report that MARCH1 modifies MHC II with a mixture of Lys63-linked, Lys11-linked and Lys48-linked ubiquitin chains, with K63 linkages being the most abundant. Second, we interrogated which E2 enzymes are required for MHC II ubiquitination, by deleting candidate E2 enzymes that have previously been reported to interact with MARCH1 or its viral homologues, K3 and K5. Our data show that, unlike for K3 or K5, none of the E2 enzymes screened is essential for MARCH1 activity, indicating functional redundancy. Finally, we aim to identify additional components of the MARCH1-MHC II ubiquitination machinery by performing a genome-wide CRISPR knockout screen for genomic regulators that alter MHC II surface expression. In summary, we are conducting an in-depth analysis of the mechanism of MHC II ubiquitination by MARCH1 E3 ligase both by using primary immune cells and engineered cell models. Our findings shed light on the mechanisms that control key molecules of the immune system.