COLLOQUIA

Wednesday

COL-01-01

PLASTICITY OF DHURRIN/DEFENCE IS REGULATED BY METHYL JASMONATE IN SORGHUM *(SORGHUM BICOLOUR)*

Sohail M.N., Blomstedt C. and Gleadow R.
School of Biological Sciences, Monash University, 3800 Victoria, Australia.

The cyanogenic glucoside, dhurrin plays an important role in growth and defence in sorghum. Previous research has shown close relationship of dhurrin with plant growth and response to herbivory but conclusive studiers were lacking. To check the role of different hormones (involved in plant growth and defence) methyl jasmonate (MeJA) and kinetin were exogenously sprayed on sorghum plants at six leaf stage. Plants were harvested at nine different time points after hormone treatment (0, 12, 24, 48, 72, 96, 120, 144, 168 h) with fifteen plants per treatment per time point. Each plant was split into half and stored for cyanide and RNA analysis at 60 °C drying oven and -80 °C freezer, respectively. To reduce the environmental variation plants were rotated twice a week before hormone application. Due to volatile nature of MeJA, both control and treated plants were covered in bags. Soon after hormone application hydrogen cyanide potential (HCNp) decreased in kinetin treated plants but this trend was also observed in control plants. HCNp started to increase after 48 h but again declined after 72 hours. The MeJA treated plants showed increase in total cyanide potential soon after 12 h of hormone application. This trend kept on increasing until 48 h and then remained almost constant, whereas control plants didn't show any change in their total cyanide potential throughout the experiment. Right now transcript levels of genes involved in dhurrin regulation are being determined by QPCR. Overall no significant effect of kinetin treatment on dhurrin concentration of sorghum was observed; however MeJA treated plants showed strong response by increased dhurrin concentration.

COL-01-02

CARBON PARTITIONING, GROWTH AND DEVELOPMENT OF A TRANSGENIC LEAF OIL CROP

Mitchell M., Pritchard, J., Okada, S., Vanhercke T., Larkin P., Petrie J., Singh S. and Ral J.-P.
CSIRO Agriculture and Food, Clunies Ross St, Acton 2601, ACT, Australia.

Demand for vegetable oil is projected to double world-wide within the next decades due to increasing food, feed, fuel and industrial requirements. Accumulation of excess photosynthetic energy in the form of leaf oil could be a solution to this growing demand. However, lipid (TAG) accumulation tends to be limited in leaves in favour of starch production. The aim of this study was to investigate the relationship between lipid, starch and soluble sugar accumulation in our unique high oil transgenic tobacco plants. In tobacco leaves, oil accumulation was correlated with greatly altered starch and soluble sugar content, although the relationship between carbohydrates and oil was highly dependent on plant and leaf developmental stage. Despite these dramatic shifts in carbon partitioning, high oil plants showed a relatively small reduction in final biomass. Evidence of diurnal cycles of TAG synthesis and degradation suggests that tobacco may be able to take advantage of this novel energy store to fuel growth at night. Further work is being undertaken to determine whether this and other mechanisms may allow tobacco to grow and accumulate oil even with such major changes to primary metabolism. In the long term, the aim of this project is to apply all knowledge gained to further increase agricultural lipid (TAG) productivity and to generate novel products for food and industry.

COL-01-03

HOST INDUCED GENE SILENCING OF *RHIZOCTONIA SOLANI* MAP KINASE GENES, RPMK1 AND RPMK2, AN EFFICIENT STRATEGY TO PRODUCE SHEATH BLIGHT RESISTANT RICE

Manoharan A.J.[1], Tiwari I.M.[2], Sharma T.R.[1] and Botella J.R.[2]
[1]Plant Genetic Engineering Laboratory, School of Agriculture and Food Sciences, University of Queensland, Brisbane, Queensland 4072, Australia. [2]National Research Centre on Plant Biotechnology, Pusa Campus, New Delhi-110012,INDIA.

Abstract *R. solani* is an economically important plant pathogen. It has a world wide distribution and is estimated to infect nearly all cultivable plants. Sheath blight disease of rice caused by *R. solani* is especially devastating, causing as much as 50% yield losses annually. Despite this threat effective strategies to control this pathogen are still lacking. In recent times, genetically modifying plants to produce RNAi triggers to silence pathogen genes, also known as Host Delivered RNAi, has emerged as a promising technology to produce pathogen resistant plants. In this work, we developed the HDRNAi technology to produce sheath blight resistant rice. Susceptible rice cultivar, Taipei-309 was transformed to encode inverted repeats that target both *R. solani* MAP kinase genes - RPMK1 and RPMK2. These genes are homologues of Magnaporthe grisea pathogenicity MAP Kinase (PMK1).*pmk1* mutants show complete loss of pathogenicity phenotype. In our study, transgenic rice lines when assayed for *R. solani*disease resistance, they show delayed disease development, reduced lesion number and lesion size as compared to wild type control. Additionally, *R. solani* infecting the transformed rice lines displayed reduced mRNA levels for both the RPMK genes - an average of 57% and 66% reduction respectively for RPMK1 and RPMK2. This is the first report demonstrating utility of HDRNAi to produce sheath blight resistant rice. **Key Words**: HD-RNAi, *Rhizoctonia solani*, Sheath blight, Disease Resistance, Taipei-309, MAP Kinase.

COL-01-04

THE TRANSCRIPTION FACTOR TRANSCRIPTOME OF WALL INGROWTH DEPOSITION IN PHLOEM PARENCHYMA TRANSFER CELLS OF *ARABIDOPSIS THALIANA*

Wu Y.[1], Hou J.[1], Yu F.[1,2], Nguyen T.[1] and McCurdy D.[1]
[1]School of Environmental and Life Sciences, The University of Newcastle, Callaghan, NSW 2308, Australia. [2]College of Forestry, Jiangxi Agricultural University, Jiangxi Province 330045, China.

Phloem parenchyma (PP) cells of *Arabidopsis* leaf veins *trans*-differentiate to become PP transfer cells (TCs) which are thought to aid phloem loading by facilitating unloading of photoassimilates into the apoplasm for subsequent energy-dependent uptake into the sieve element/companion cell (SE/CC) complex. We are using PP TCs in Arabidopsis as a genetic model to identify transcription factors putatively involved in coordinating the deposition of the wall ingrowth network. Detailed analysis of wall ingrowth deposition by confocal microscopy of modified pseudo-Schiff-propidium iodide-stained tissue shows that wall ingrowths are absent in PP cells of 5-day-old cotyledons but abundant in cotyledons at 10 days, and similarly absent in leaf 1 of 10-day-old seedlings but abundant in leaf 1 at 16 days. Using these observations, we have undertaken transcript profiling (RNA-Sequencing) of wall ingrowth deposition in PP TCs and identified 41 differentially expressed (FDR-corrected *P* values ≤0.05) transcription factors that are commonly up- or down-regulated when comparing 5-day vs 10-day cotyledons, 10-day vs 16-day leaf 1, and 10-day cotyledons vs 10-day leaf 1. Among them, the 22 transcription factors commonly up-regulated were characterized by members of the NAC-domain, MYB and ERF families. Several of the NAC-domain transcription factors, including the paralogs*AtNAC56* and *AtNAC18* have been identified as genes involved in secondary wall formation. We report results from co-expression network analysis to further refine a subset of transcription factors likely to participate in genetic regulation of wall ingrowth deposition and test these predictions by phenotypic analysis of relevant mutants.

COL-01-05

REGULATION OF MICRORNAS FOR THE PRODUCTION OF ADVENTITIOUS ROOTS

Gleeson M.[1], Hayward A.[1], Beveridge C.[1], Carroll B.[1], Lee Y.[2], Ware D.[2] and Mitter N.[1]
[1]The University of Queensland. [2]Cold Spring Harbour Laboratories.

Many horticultural industries, such as that of avocado, are constrained by woody-tree recalcitrance towards adventitious root production for clonal propagation. Consequently, it is pertinent to develop our understanding of the processes regulating adventitious rooting. MicroRNAs make excellent candidates for such studies, as they are highly conserved across the plant kingdom and are central regulators to many developmental pathways. In this study, miR160, miR167 and their AUXIN RESPONSE FACTOR regulatory targets, known to be involved in adventitious root production, have been studied in both Arabidopsis and avocado (*Persea americana*). New potential players in the adventitious rooting pathway involving these genes are being examined using Arabidopsis knock-out lines of transcription factors shown to interact with their promoter regions. Lines found to have significantly altered adventitious rooting phenotypes compared to wild-type controls were selected for further phenotyping and molecular characterisation of these microRNAs and their targets. Analysis of microRNA activity in avocado material undergoing traditional industry clonal propagation protocols will also be presented. Strengthening our understanding of adventitious rooting by studying microRNAs has potential to drive improvements to clonal propagation in our important horticultural crops.

COL-01-06

THE 5' UNTRANSLATED REGION (UTR) OF A CAROTENOID MESSENGER RNA CAN SENSE METABOLITE ACCUMULATION IN ARABIDOPSIS

Aryal R.[1], Alagoz Y.[1], Holland S.[2], Watkins J.[2], Shashikanth M.[2], Pogson B.[2] and Cazzonelli C.[1]
[1]Hawkesbury Institute for the Environment, Western Sydney University. [2]Research School of Biology, Australian National University.

Many messenger RNAs, in addition to protein coding regions, harbor regulatory domains in their untranslated regions (UTRs). In bacteria, several families of RNA structural switches called riboswitches are found in the UTRs of mRNA that sense metabolite accumulation and regulate gene or protein expression. In eukaryotes however, only the thiamine riboswitch has been identified to date. Metabolic pathways are tightly controlled in plants in order to maintain cellular homeostasis. Arabidopsis epsilon lycopene cyclase (εLCY) expression is responsive to cis-carotenoid feedback regulation (Cazzonelli et al. 2009, Plant Cell 21:39-53). Here we describe the metabolic feedforward regulation by an RNA regulatory switch at the 5' UTR of the Arabidopsis εLCY gene. The Arabidopsis εLCY harbors two alternative transcription start sites separated by 74bp AT rich segment. The longer transcript can form two alternative secondary structures at the 5' end. The 74bp additional sequence in the longer transcript is highly conserved across many plant species indicating its importance in gene regulation. Presence of the shorter transcript suggests that the 74bp terminal sequence is dispensable and actively removed in response to specific environmental conditions. The εLCY expression is downregulated in the Arabidopsis and tomato mutants that accumulate cis-carotenoid intermediates. We created a reporter gene construct with εLCY promoter plus 5' UTR fused with luciferase. The expression of luciferase is downregulated in Arabidopsis etiolated seedlings accumulating cis-carotenoids indicating a role of the 5' UTR and promoter in sensing carotenoid flux through the pathway. Our findings highlight the importance of the 5' UTR in sensing metabolic change in plants.

COL-02-01

IDENTIFYING CANCER STEM CELLS IN TUMOURSPHERES

Schoning J.P., Monteiro M. and Wenyi G.
Australian Institute for Bioengineering and Nanotechnology (AIBN), University of Queensland (UQ), Corner College and Cooper Roads, St Lucia, Brisbane QLD, Australia.

Effective cancer treatment is hindered by a small subset of cells called cancer stem cells (CSCs), which are responsible for chemotherapy resistance and metastasis. In order to study CSCs tumoursphere culture is commonly used to enrich CSCs in vitro. However, not all cells in tumourspheres are CSCs, therefore, clear characterisation and isolation methods are necessary. Importantly, due to the diversity of cancer tissues, there are no universal molecular markers for CSCs. There is also little consensus on CSC surface markers within commercially available cell lines, such as colon cancer cell line HCT116, with ambiguity surrounding the relevance of proposed CSC, markers including CD133. This uncertainty may arise from using only surface markers as primary identifiers of CSCs rather than fundamental growth characteristics of CSCs. Here we used a fluorescent label retaining assay, commonly used to identify stem cells in neurospheres, to isolate potential CSCs based on their slow growth (quiescent) characteristics. Preliminary data demonstrates negligible overlap between the label retain cells (LRCs) and the CD133 surface marker. This indicates that CD133 may not designate quiescent CSCs. Other molecular markers were explored, including the OCT4B protein. Confocal analysis demonstrated differential cellular localisation and expression levels of OCT4B in HCT116 cells. Subsequent characterisation of OCT4B expression in LRCs using flow cytometry and whole sphere confocal microscopy will determine the functionality of using OCT4B as a marker for CSC isolation in tumourspheres. Finding relevant molecular markers would enable meaningful monitoring of CSCs in tumoursphere culture and will provide more information in the development of targeted CSCs treatments.

COL-02-02

THE ROLE OF GRAINYHEAD-LIKE 3 (GRHL3) IN THE REGULATION OF GASTRULATION, AXIAL FORMATION AND EARLY SKIN DEVELOPMENT

Miles L.B.[1], Tang J.[1], Kaslin J.[1], Jane S.M.[1], Heath J.K.[2] and Dworkin S.[1, 3]
[1]Monash University, Clayton Campus, Melbourne, VIC, 3800. [2]Walter and Eliza Hall Institute, 1G Royal Pde, Parkville, VIC, 3050.[3]La Trobe University, Bundoora, VIC, 3086.

The highly conserved transcription factor grainyhead-like 3 (grhl3) is a critical regulator of embryogenesis, involved in formation of the neural tube, closure of the secondary palate, development of the epidermal barrier, and playing an important role in wound healing. We are investigating the role for the grhl3 transcription factor during development, using the highly-tractable zebrafish as our model. Our preliminary data show that grhl3 is strongly and specifically activated at the maternal-zygotic transition (MZT) preceding gastrulation, and subsequently that grhl3 is required for EVL/periderm formation and maintenance. Our preliminary results indicate that both loss and overexpression of grhl3 leads to severe problems during gastrulation, resulting in reduced epiboly, a split trunk/axis, reduction in body size, defects in EVL cell formation, and craniofacial malformations. We are currently generating null mutant grhl3 zebrafish (using CRSIPR/Cas9 genome modification) to extend our preliminary findings, as well as a transgenic line capable of inducible grhl3 overexpression after heat shock treatment. Using advanced molecular and bioinformatics approaches, our current focus is on identifying novel grhl3 target genes, protein binding partners, and downstream signalling pathways which operate during early embryonic development.

COL-02-03

DISSECTING THE MOLECULAR FUNCTION OF THE MAMMALIAN MALE SEX-DETERMINING FACTOR SRY

Zhao L.[1], Ng E.T.[1], Quinn A.[1], Veyrunes F.[2], Longmuss E.[1], Moisyadi S.[3], Bowles J.[1, 4] and Koopman P.[1]
[1]Institute for Molecular Bioscience, The University of Queensland. [2]Université Montpellier II, France. [3]University of Hawaii, USA. [4]School of Biomedical Sciences, The University of Queensland.

The Y-linked mammalian male sex-determining gene *Sry* encodes a DNA-binding transcription factor. Once expressed in the gonadal primordium, SRY binds to its target enhancer TESCO of *Sox9* and activates its expression, which in turn leads to Sertoli cell differentiation and testis development. How SRY activates *Sox9* expression remains poorly understood, as SRY lacks a recognizable transactivation domain. Using combined *in vivo* and *in vitro* approaches, we demonstrate that mouse SRY has evolved a novel bifunctional module essential for both SRY protein stabilisation and transactivation of *Sox9*. We further analysed the structure and function of SRY in *Mus minutoides*, a mouse species where XY individuals bearing a variant X chromosome develop naturally as females. We found that the sequence encoding the bifunctional module is severely degraded, which results in an almost completely abolished ability of SRY to activate *Sox9*-TESCO from closely related laboratory mice. Based on these data, we suggest that evolution of the SRY bifunctional module has played an important role in mouse speciation and the evolution of unusual sex determination systems.

COL-02-04

CELL TYPE-SPECIFIC EXPRESSION OF NFIX IN THE DEVELOPING AND ADULT CEREBELLUM

Fraser J.M.[1], Essebier A.[2], Mutemi K.[1], Gronostajski R.M.[5], Boden M.[2], Wainwright B.J.[3], Harvey T.J.[1] and Piper M.[1, 4]
[1]The School of Biomedical Sciences, University of Queensland. [2]The School of Chemistry and Molecular Biosciences, University of Queensland. [3]The Institute for Molecular Bioscience, University of Queensland. [4]Queensland Brain Institute. [5]Department of Biochemistry, Program in Genetics, Genomics and Bioinformatics, Center of Excellence in Bioinformatics and Life Sciences, State University of New York at Buffalo.

Transcription factors from the Nuclear Factor One (NFI) family have been shown to play a central role in regulating neural progenitor cell differentiation within the embryonic and postnatal brain. NFIA and NFIB, for instance, promote the differentiation and functional maturation of granule neurons within the cerebellum. Mice lacking *Nfix* in all cells exhibit delays in the development of neuronal and glial lineages within the cerebellum, but the cell type-specific expression of this transcription factor remains undefined. We have examined the expression of NFIX, in conjunction with various cell type-specific markers, within the developing and adult cerebellum using co-immunofluorescence labelling and confocal microscopy. Embryonically, NFIX was expressed by progenitor cells within the rhombic lip and ventricular zone. Postnatally, progenitor cells within the external granule layer, as well as migrating and mature granule neurons, expressed NFIX. Within the adult cerebellum, NFIX displayed a broad expression profile, and was evident within granule cells, Bergmann glia and interneurons, but not within Purkinje neurons. Furthermore, transcriptomic profiling of cerebellar granule neuron progenitor cells showed that multiple splice variants of *Nfix* are expressed within this germinal zone of the postnatal brain. Collectively, these data suggest that NFIX plays a role in regulating progenitor cell biology within the embryonic and postnatal cerebellum, as well as an ongoing role within multiple neuronal and glial populations within the adult cerebellum.

COL-02-05

THE MICROCEPHALY GENE WD40-REPEAT PROTEIN 62 (WDR62) REGULATES CILIA FORMATION, CHEMO- AND PHOTO-SENSATION IN DROSOPHILA MELANOGASTER

Shohayeb B.[1], Quinn L.[2], Millard S.[1] and Ng D.[1]
[1]School of Biomedical Science, University of Queensland, St Lucia, Queensland 4067, Australia. [2]School of Biomedical Science, University of Melbourne, Parkville, Victoria 3010, Australia.

Cilia are microtubule-based structures (axonemes) that extend from centrioles (basal bodies), which are also principal components of centrosomes. Cilia project from the cell body and perform critical sensory and signalling functions essential for normal development and tissue homeostasis. Ciliary defects cause a myriad of diseases in humans collectively known as ciliopathies. WD40-Repeat Protein 62 (WDR62) is a centrosome and microtubule-associated signalling protein that is genetically linked to the autosomal recessive condition of primary microcephaly. Interestingly, wdr62 mutations cause CNS malformations, behavioural and learning disorders that phenotypically overlap ciliopathies. The molecular and cellular basis of WDR62 functions in brain formation, and whether this involves regulation of the primary cilium remains undefined. Our current study utilized fly genetics to investigate whether the Drosophila wdr62 ortholog (CG7337) was required for cilia formation and function in sensory neurons that innervate chemo- and mechano-sensory bristles in the legs, wings and antennae. We visualized ciliated sensory neurons using neural specific (Elav Gal4) mCD8-GFP expression and observed increased cilia length in the mechanosensory neurons of campaniform sensilla in the wing vein following CG7337 knockdown. Interestingly, cilia formation was deficient in mechanosensory neurons in femoral chordotonal groups in the legs, as well as in the chemosensory neurons in the antennae. Behavioural assays revealed defects in phototaxis and chemosensory responses of third instar larvae consistent with cilia defects observed in adult flies. Thus, our studies show that wdr62/CG7337 has an essential role in regulating cilia formation, which may have implications for mammalian ciliogenesis.

COL-02-06

HOW IS MEIOSIS INITIATED IN THE MOUSE FETAL OVARY?

Feng C.A.[1, 2], Davidson T.[2], Koopman P.A.[2] and Bowles J.[1, 2]
[1]School of Biomedical Sciences, The University of Queensland, Brisbane, Australia. [2]Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia.

There is a population of cells in the gonads called the germ cells that are the precursors of spermatids and oocytes and, as such, they possess the unique ability to undergo meiotic cell division in order to generate these haploid gametes. In mammals, a factor critical for the initiation of meiotic entry is Stimulated by Retinoic Acid gene 8 (STRA8) and, as the name suggests, retinoic acid (RA) is a known regulator of the associated gene. It is now well-accepted that RA is required for *Stra8* expression in the post-natal testis and substantial evidence exists that, during fetal ovarian development, RA induces germ cells to express *Stra8*. Nonetheless, the role of RA in the fetal ovary has been disputed principally because some germ cells in embryos lacking two major RA-synthesising enzymes, ALDH1A2 and ALDH1A3, remain able to enter meiosis. Another major factor casting doubt over the involvement of RA in the fetal ovaries is that studies utilizing Stra8 promoters containing putative RA responsive elements are only capable of driving transgene expression in the post-natal testis and not fetal ovaries. New evidence for the role of RA in initiating *Stra8* expression will be presented. We have also studied the specific molecular mechanisms involved in the onset of*Stra8* expression focusing on a previously unexamined region of the *Stra8* promoter and identified candidate regulatory elements using *in vitro* assays. Going further we have confirmed the function of the identified element(s) in regulating ovarian meiosis through direct genome editing of the genomic *Stra8* promoter using CRISPR/Cas9 technology in mice.

COL-03-01

MECHANISM OF ACTION OF ARTEMISININ ANTIMALARIALS AND IMPLICATIONS FOR DRUG RESISTANCE IN *PLASMODIUM FALCIPARUM*

Xie S.C.[1], Dogovski C.[1], Burgio G.[2], Foote S.[2], Klonis N.[1] and Tilley L.[1]
[1]Department of Biochemistry and Molecular Biology, Bio21 Institute, The University of Melbourne, Melbourne, VIC 3010, Australia.[2]John Curtin School of Medical Research, The Australian National University, Canberra, ACT 0200, Australia.

Current first-line artemisinin antimalarials (ARTs) are threatened by the emergence of resistant *Plasmodium falciparum.* ART resistance has recently been linked to mutations in the K13 propeller protein. Our characterization of field strains (Pailin, Cambodia) shows that decreased sensitivity of ART resistant parasites is evident in the initial (early ring) stage of intraerythrocytic development, making it critical to understand the action of ARTs at this stage. We show that a specific hemoglobinase inhibitor (E64d) strongly antagonizes ART action at ring stage of the 3D7 strain, indicating a major role of heme in ART activation. The surprising implication that hemoglobin digestion is active in early rings is supported by pulldown-based identification of active hemoglobinases (falcipains) at this stage. We show that genetic down-modulation of the expression of the two main cysteine protease hemoglobinases, falcipains 2 and 3, renders early ring stage 3D7 parasites resistant to ARTs. This shows that changes in the rate of ART activation could mediate high levels of ART resistance. Our kinetic analysis of the K13 wildtype and mutant isolates reveals that exposure to short pulses of ARTs induces growth retardation in both sensitive and resistant parasites. Following this growth retardation, resistant strains survive, while sensitive parasites succumb. The data suggest that ARTs are activated, and cause cellular damage, in both strains, but resistant parasites are better able to withstand the damage. Since arrest in growth often reflects the cellular stress response, we postulated that ART resistance is caused by an up-regulated parasite cellular defense mechanism. Consistent with this, we demonstrate that proteasome inhibitors effectively synergize ART activity against both sensitive and resistance strains, with particularly strong synergism evident during the most resistant stage of the resistant strains. This suggests that the parasite proteasome system could be targeted to enhance drug action, offering a way to overcome ART-resistant malaria.

COL-03-02

NUCLEAR TRAFFICKING OF VENEZUELAN EQUINE ENCEPHALITIS VIRUS CAPSID PROTEIN AS AN ANTIVIRAL TARGET

Thomas D.R.[1], DeBono A.[2], Lundberg L.[3], Pinkham C.[3], Shechter S.[4], Kehn-Hall K.[3], Baell J.[2], Wagstaff K.M.[1] and Jans D.A.[1]
[1]Department of Biochemistry and Molecular Biology, Monash University, Clayton, Vic, Australia. [2]Department of Medicinal Chemistry, Monash University, Parkville, Vic, Australia. [3]National Center for Biodefence and Infectious Diseases, School of Systems Biology, George Mason University, Manassas, VA, USA. [4]Shechter Solutions, Andover, MA, USA.

Venezuelan Equine Encephalitis Virus (VEEV) is an *Alphavirus* that can be fatal to either humans or equines, but there is no widely available treatment, and the only available vaccine has significant side effects and limited efficacy. The VEEV capsid protein is critical to VEEV biology, both encasing the viral RNA, and acting to down-regulate host antiviral defenses by inhibiting nuclear transport in the infected host cell. Capsid contains targeting signals for both nuclear import and export, with viruses containing mutations in either of these signals strongly attenuated. Capsid is thought to inhibit host cell nuclear transport by binding to and sequestering 3 members of the importin (IMP) superfamily of nuclear transport proteins (IMPα, IMPβ1, Crm1) within the nuclear envelope that surrounds the nucleus. Using fluorescence recovery after photobleaching (FRAP) in cells transfected to express capsid, we have shown that even though capsid localizes strongly at the nuclear envelope, it is able to translocate readily into and out of the nucleus, and exhibits dynamic localization at the nuclear envelope itself, Using high throughput screening and*in silico* approaches, we have identified and begun to characterize compounds that inhibit the capsid:IMPαβ1 interaction. Preliminary results suggest that these compounds both reduce the rate of capsid nuclear import, and show antiviral activity against VEEV in a cell culture infectious model. These compounds are exciting prospects as VEEV antivirals to be developed through medicinal chemistry in the future.

COL-03-03

RECRUITMENT OF ADAPTOR PROTEIN COMPLEX 4 BY SMALL G PROTEIN ARL5B AT THE *TRANS*-GOLGI NETWORK TO REGULATE THE ANTEROGRADE TRAFFICKING OF β-AMYLOID PRECURSOR PROTEIN

Tan J.Z.A., Toh W.H., Houghton F.J. and Gleeson P.A.
The Department of Biochemistry and Molecular Biology and Bio21 Institute, The University of Melbourne, Victoria 3010, Australia.

Alzheimer's disease (AD) is characterized by the extracellular deposition of amyloid plaques in the brain. These plaques are formed by the accumulation and aggregation of β-amyloid peptide (Aβ) derived through the sequential cleavage of β-amyloid precursor protein (APP) by membrane-bound enzymes β-secretase (BACE1) and γ-secretase, with BACE1 cleavage being the rate-limiting step in Aβ production. The levels of Aβ production have been shown to be dependent on the subcellular localization and trafficking pathways of APP and BACE1. Although the processing of APP has been shown to occur in the secretory pathway, the post-Golgi trafficking of APP is not well understood. Adaptor protein complex 4 (AP-4) has recently been implicated in the post-Golgi trafficking of APP. However, it has been unclear how AP-4 is recruited to the *trans*-Golgi network (TGN). Here we have identified Arl5b, an Arf-like (Arl) small G protein, to play a role in the TGN recruitment of AP-4. AP-4 has been found to co-immunoprecipitate with active GTP-bound Arl5b, suggesting a potential interaction. The knockdown of Arl5b in cultured cells results in a reduction in TGN localisation of AP-4 but not AP-1 (another TGN localized AP complex), accumulation of APP at the TGN, and increased Aβ production. AP-4 mutations have been discovered in human patients suffering from severe brain abnormalities, thus we now seek to determine the role of AP-4 in the post-Golgi trafficking of APP in neurons. Collectively, we propose that an efficient post-Golgi trafficking of APP is critical in regulating Aβ production.

COL-03-04

P. MIRABILIS SCSC: A TRIMERIC, SHAPE-SHIFTING DISULFIDE ISOMERASE

Furlong E.J.[1], Kurth F.[1], Premkumar L.[1], Choudhury H.G.[1] and Martin J.L.[1, 2]
[1]Institute for Molecular Bioscience, The University of Queensland, St Lucia, QLD 4072, Australia. [2]Eskitis Institute for Drug Discovery, Griffith University, Nathan, QLD 4111, Australia.

Bacterial disulfide isomerases are typically homo-dimers, which shuffle incorrect disulfide bonds in their protein targets. These enzymes are essential for the correct folding of proteins required for bacterial virulence and survival under stress. *Proteus mirabilis*ScsC (PmScsC) is a disulfide isomerase that surprisingly, functions as a trimer. We have solved the crystal structure of PmScsC in three different conformations. The major conformational difference observed in these structures is the distance between the trimerisation domain and the catalytic domain. In the compact structure the catalytic domains of all three protomers pack close to the trimerisation domain, in the extended structure the catalytic domains are much further away from the trimerisation domain, while the transitional structure has features of both other structures. Comparison of these structures reveals a flexible linker in the protein, which changes secondary structure to facilitate the conformational changes. The structures also show that *P. mirabilis*ScsC has a large range of motion and we predict that this is necessary for its disulfide bond shuffling activity.

COL-03-05

STRUCTURAL AND BIOCHEMICAL CHARACTERISATION OF *CHLAMYDIA TRACHOMATIS* DSBA REVEALS A CYSTEINE-RICH AND WEAKLY OXIDISING OXIDOREDUCTASE

Christensen S.[1], Grøftehauge M.[1], Byriel K.[1], Heras B.[2], McMahon R.[1] and Martin J.L.[1]
[1]Division of Chemistry and Structural Biology, Institute for Molecular Bioscience, University of Queensland, St. Lucia, Queensland 4072, Australia. [2]La Trobe Institute for Molecular Science, La Trobe University, Melbourne, VIC 3086, Australia.

The rise of antibiotic drug resistance is undermining our ability to treat an increasing range of bacterial infections and is a huge threat to public health worldwide (1) Disulfide bonds provide structural bracing to numerous proteins including those involved in bacterial pathogenesis (2). The bacterial disulfide bond protein DsbA catalyses the formation of disulfide bonds and is thus a potential target for drug development. The focus of this study is DsbA from the obligate intracellular human pathogen *Chlamydia trachomatis* (CtDsbA). CtDsbA stands out from other DsbA enzymes by having an uneven number of cysteines, an additional disulfide bond as well as an uncommon dipeptide sequence in the catalytic motif (CSAC). We report the 2.7Å crystal structure of CtDsbA revealing a typical DsbA fold. This study confirms that CtDsbA has oxidase activity and redox properties similar to other DsbAs. However, DsbA is a significantly weaker oxidase than other DsbAs studied. This can be explained by a lack of factors stabilizing the active site nucleophilic thiolate. The characterization of CtDsbA contributes to the broader understanding of the redox properties of DsbA proteins and supports ongoing efforts to develop inhibitors of these proteins. (1) Beating bad bugs. Nat Rev Drug Discov, 2010. 9(9): p. 663. (2) Bardwell, J.C., K. McGovern, and J. Beckwith, Identification of a protein required for disulfide bond formation in vivo. Cell, 1991. 67(3): p. 581-9.

COL-03-06

STRUCTURAL BASIS OF ZN(II) ACQUISITION MECHANISM IN PNEUMOCOCCUS

Luo Z.[1, 2, 3], Morey J.[4], McEwan A.[1, 2], McDevitt C.[4] and Kobe B.[1, 2, 3]
[1]School of Chemistry and Molecular Biosciences, University of Queensland. [2]Australian Infectious Diseases Research Centre, University of Queensland. [3]Institute for Molecular Biosciences, University of Queensland. [4]Research Centre for Infectious Diseases, School of Biological Sciences, University of Adelaide.

Pneumococcus (Streptococcus pneumoniae) is among the world's leading bacterial pathogens and is responsible for over 1 million deaths annually. Transition metals, such as Zn(II), have been recognized as essential nutrients for the growth and virulence of pathogenic bacteria in humans. Acquisition of Zn(II) in pneumococcus is supported by the ATP binding cassette transporter AdcBCA, which includes a surface protein AdcA as the initial receptor for Zn(II) recognition. AdcA is a high affinity Zn(II)-specific substrate binding protein with two Zn(II)-binding domains (ZnuA-domain and ZinT-domain) and its imperative role in the survival and pathogenicity of the pneumococcus makes the protein a potential therapeutic target for the development of novel antimicrobial drugs against pneumococcal diseases. However, the lack of experimentally determined structures of AdcA hinders a deeper understanding of the Zn(II) acquisition mechanism in the pneumococcus. Therefore, in this study, we determined a series of high-resolution crystal structures of AdcA and its individual domains by X-ray crystallography. In the structures, both of the AdcA domains possess Zn(II)-binding sites and are capable of capturing Zn(II) with high affinity. Also, the two domains can interact with each other and form a tract of histidine residues connecting their Zn(II)-binding sites. Based on the structures, we hypothesize that the two domains in AdcA work collaboratively to acquire Zn(II) with high efficiency during severe Zn(II) shortage in hosts. The results provide the first structural insights into the mechanism of AdcA-mediated Zn(II) acquisition in the pneumococcus.

COL-04-01

PHENOTYPE SWITCHING IN MELANOMA PROGRESSION

Simmons J.L., Pierce C.J., Al-Ejeh F. and Boyle G.M.
QIMR Berghofer Medical Research Institute.

Metastatic melanoma is an aggressive disease associated with poor survival, owing to a high level of therapeutic resistance. Phenotypic plasticity is an occurrence whereby tumour populations switch between proliferative and invasive states, also termed phenotype switching. It has been suggested that the aggressive nature and inherent chemotherapeutic resistance of metastatic melanoma is a result of this plasticity. Recent studies have shown mutually exclusive expression of the transcription factors MITF and BRN2 in metastatic melanoma tumours. Cells within proliferative regions of a tumour display high expression of MITF while invasive regions and disseminated tumour cells have increased expression of BRN2. This has lead to the suggestion that MITF and/or BRN2 may be contributing to phenotype switching in metastatic melanoma. Utilising both *in vitro* and *in vivo* methods we have investigated the relative contribution of these two transcription factors towards phenotype switching leading to melanoma metastasis.

COL-04-02

BASAL CELL CARCINOMA DEVELOPMENT IS PROMOTED BY ABLATION OF THE DERMAL PAPILLA HAIR FOLLICLE MESENCHYMAL NICHE

Villani R.M.[1, 2], Sim S.L.[1], Greaney J.[2], Wainwright B.J.[3] and Khosrotehrani K.[1, 2]
[1]Diamantina Institute, UQ, Translational Research Institute, 37 Kent St, Woolloongabba, Brisbane. [2]UQ Centre for Clinical Research, UQ, RBWH Campus, Herston, Brisbane. [3]Institute for Molecular Bioscience, 306 Carmody rd, St Lucia, Brisbane.

Basal Cell Carcinoma (BCC) is an extremely common skin tumour that relies very heavily on its environment. Despite its importance, little is currently known about the tissue surrounding the BCC or its niche, neither the cellular origin nor the signal mechanisms resulting in its development. By studying the niche we propose that we can both develop better therapeutics and also prevent the establishment of permissive environments for secondary lesion development. We therefore investigated the role of the dermal papilla, a specific type of skin fibroblast, in the BCC niche. The dermal papilla has essential roles in maintaining hair follicle proliferation and we have shown in mice that dominant negative mutation of Sox18 inhibits the differentiation of dermal papilla. Using this model we have combined dominant negative Sox18 mutation with a murine BCC model, and have deleted dermal papilla from the BCC niche. Interestingly, deletion of the dermal papilla lineage promotes BCC development, along with increased epidermal proliferation and progenitor markers such as Sox9. These results indicate that the dermal papilla negatively regulates BCC development. Our hair follicle studies also indicate that loss of associated dermal papilla induces exit from the stem cell compartment in hair follicles and therefore increased BCC development after Sox18 dominant negative mutation is likely due to the promotion of the epidermal progenitor compartment. This study gives precedence that modulation of the BCC niche can affect tumour development and furthermore induction of DP-like signalling in the BCC niche may represent a new avenue for investigation in developing BCC therapeutics.

COL-04-03

IDENTIFICATION OF A NOVEL LONG NON-CODING RNA AT THE *5P15* LOCUS ASSOCIATED WITH PROSTATE CANCER RISK

Panchadsaram J.[1, 2], Tevz G.[1, 2], Stylianou N.[1, 2], Hollier B.[1, 2], Nelson C.[1, 2], The Australian Prostate Cancer Bioresource [1], Williams E.D.[1, 2], Clements J.[1, 2] and Batra J.[1, 2]

[1]Institute of Health and Biomedical Innovation, School of Biomedical Sciences, Queensland University of Technology, Brisbane, Australia. [2]Australian Prostate Cancer Research Centre Queensland, Princess Alexandra Hospital, Translational Research Institute, Brisbane, Australia.

Prostate Cancer (PCa) is the second most common cause of cancer death in Australian men. Genome-Wide Association Studies (GWAS) have provided insight into genomic regions that alter an individuals risk of developing PCa and shortlisted 100 risk loci. Through the GWAS, chromosome *5p15* has been identified as a PCa risk associated locus in multi-ethnic populations. *IRX4* has been previously identified as the top-ranked expression quantitative trait locus (eQTL) at *5p15*. In addition, we discovered a long non-coding RNA *(IRX4lncRNA)* in the anti-sense strand of *IRX4* using paired-end RNAseq. This lncRNA was overexpressed in prostate tumor samples compared to their adjacent non-malignant tissues (n=50). Knockdown of lncRNA reduced proliferation of LNCaP cells, implicating its role in promoting PCa growth. Furthermore, we found an up-regulation of *IRX4lncRNA* in cells undergoing mesenchymal to epithelial transition, a hallmark of PCa invasion. As androgens promote PCa growth, we tested the effect of androgen ablation therapy on *IRX4lncRNA*. Surprisingly, it was up-regulated by androgens in VCaP cells and down-regulated in LNCaP cells. Data-mining revealed binding of two crucial transcription factors, AR and ERG, at this locus in VCaP cells, whereas no AR binding was observed in ERG negative LNCaP cells. We also noted a correlation between *IRX4lncRNA*expression and ERG fusion in our RNA-sequencing data from a cohort of seven androgen-responsive patient-derived xenografts. Further functional characterisation of this novel *IRXlncRNA* will clarify its therapeutic potential in PCa pathogenesis.

COL-04-04

DESIGNING BIASED LIGANDS FOR PROTEASE ACTIVATED RECEPTOR 2

Suen J.Y., Jiang Y., Yau M.K., Lim J., Kok W.M., Xu W. and Fairlie D.P.
Institute for Molecular Bioscience, The University of Queensland, St. Lucia, QLD, Australia.

Protease Activated Receptor 2 (PAR2) has been implicated in inflammation, cancer and metabolic disorders. The receptor mediates communication between extracellular proteases and intracellular signaling pathways. Increasing numbers of proteases have been reported to activate PAR2 with different signaling profiles, suggesting different physiological portfolios. Such 'biased' signaling could bring benefits to the cell while modulating other disease-associated pathways. However, to date there has not been a systemic approach to understanding and developing biased ligands for this receptor. We have performed a structure-function relationship study on peptides related to the PAR2 tethered ligand, SLIGRL-NH2, with greatly improved functional potencies. We show here quantitative data for receptor binding, intracellular calcium mobilization and ERK1/2 phosphorylation for peptide agonists with single or double alanine substitutions, with the objective to understand key ligand-receptor interactions required for PAR2 biased signaling. Other chemical modifications were also explored with compounds showing single digit nM potencies. In summary, we have identified key residue crucial for agonist affinity, and a separate residue as key for dictating receptor biased signaling. We also report a few PAR2 agonists with pathway selective properties, and application of these ligands have demonstrated the importance of ERK1/2 in PAR2-induced migration, prevention of cell death and wound healing. These findings highlight the potential of potent, pathway-selective ligand of PAR2 in human diseases. Reference: Hollenberg et al. (2014) Br J Pharmacol 171:1180 Suen et al. (2014) Br J Pharmacol 171:4112 Funding: We thank the ARC and NHMRC for research funding.

COL-04-05

ACTIVATION OF RIG-I BY HAIRPIN RNA

Shah N.[1], Beckham S.A.[1], Luo D.[2], Wilce M.C.J.[1] and Wilce J.A.[1]
[1]Biomedicine Discovery Institute, Department of Biochemistry and Molecular Biology, Monash University, Wellington Road, Clayton VIC 3800, Australia. [2]Nanyang Technological University, Singapore.

As part of our innate immune system, ATP dependent RIG-I like receptors (RLRs) within the host cytoplasm detect viral RNA. RIG-I (retinoic acid inducible gene-I receptor), in particular, senses dsRNA with a 5'-triphosphate overhang and, following a conformational rearrangement and release of its CARD domains, mediates the ultimate induction of type I interferons and pro-inflammatory cytokines. It has been shown in activation assays that a 5' triphosphate-10mer dsRNA hairpin is able to activate the RIG-I, but a 5' ppp-8mer does not (1). There is no biophysical evidence yet, however, of CARD release by the 10mer dsRNA and not the 8mer. Based on the question whether 10mer is the minimum length of dsRNA enough for RIG-I activation or 8mer dsRNA can also activate it, we are investigating this using size-exclusion chromatography-coupled small-angle X-ray scattering (SAXS) (2), and limited tryptic digest experiments. In addition, by using different ATP analogues, we are examining the importance of ATP on the conformational changes of RIG-I:RNA complexes. This study will help in better understanding of the molecular interactions necessary for RIG-I activation. **REFERENCES** 1. Kohlway, A., Luo, D., Rawling, D. C., Ding, S. C., and Pyle, A. M. (2013) Defining the functional determinants for RNA surveillance by RIG-I. EMBO reports 14, 772-779. 2. Beckham, S. A., Brouwer, J., Roth, A., Wang, D., Sadler, A. J., John, M., Jahn-Hofmann, K., Williams, B. R., Wilce, J. A., and Wilce, M. C. (2013) Conformational rearrangements of RIG-I receptor on formation of a multiprotein:dsRNA assembly. Nucleic acids research 41, 3436-3445.

COL-04-06

IRAK3 MODULATES NFKB THROUGH ITS GC ACTIVITY

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

Interleukin-1 receptor associated kinase 3 (IRAK3) acts as a negative regulator of inflammation by inhibiting inflammatory downstream signaling and is involved in a number of inflammatory related diseases. IRAK3 is proposed to be useful as a diagnostic and prognostic marker in inflammation, and possibly a target for intervention. In cancer, IRAK3 is a critical mediator involved in the cross talk between tumor cells and macrophages. The exact mechanism of action and the selectivity of IRAK3 is however still largely unclear and further evaluation is needed. Our prior studies using bioinformatic search tools identified IRAK3 as a potentially novel guanylate cyclase (GC) catalyzing cyclic guanosine monophosphate (cGMP) synthesis, IRAK3 was shown to contain a GC centre within its kinase domain. We demonstrate that wild type IRAK3 is capable of producing cGMP, and that point mutations in the GC centre reduced cGMP production. cGMP alone affects downstream signaling through NFkB modulation in the presence of lipopolysaccharides. Wildtype IRAK3 functions to reduce lipopolysaccharide stimulated NFkB in cells and we have confirmed this role, however unlike wild type IRAK3, the mutant did not reduce NFkB activity. We believe that the cGMP produced may be involved in IRAK3s regulatory function where cGMP may affect selectivity in downstream signaling pathway(s) by modulating the binding and/or activity of nearby interacting proteins involved in the cascade. Our findings may provide insight into the selectivity and functionality of IRAK3 in the inflammatory signaling cascade.