SYMPOSIA

Monday – Thursday
HORMONAL REGULATION OF DIFFERENTIAL CELL GROWTH

Benkova E.
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Differential cell growth enables flexible plant development in the presence of environmental fluctuations, such as organ bending after light or gravity stimuli. A prominent example of such a differential cell growth in plants is the formation of apical hooks that protect the fragile shoot apical meristems when they penetrate the soil right after germination. Asymmetric auxin distribution (auxin gradient) coordinates the apical hook development, and its formation largely depends on the concerted action of auxin transporters including PIN-FORMED (PIN) auxin efflux carriers. Although the role of auxin transporters in the apical hook development has been well studied, detailed mechanisms underlying formation and developmental transition of auxin gradients into the differential cell growth remain elusive. Here, we combined in silico and in vivo approaches to infer a minimal mechanism underlying auxin gradient-guided differential growth during establishment of apical hooks in the model plant Arabidopsis thaliana. We propose a mechanism that translates this maximum into differential growth, and thus curvature, of the hook that was experimentally validated. Our model assumes a tight interplay between the PIN-dependent polar auxin transport and auxin-mediated cell growth dynamics. Our combined experimental and modeling studies also reveal that the hook curvature degree is determined by both auxin-mediated differential cell growth and spatial pattern of cell proliferation that involve hormonal crosstalk.

SYM-01-02

AUXINS OR ETHYLENE - WHO CONTROLS GRAPE BERRY RIPENING?

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Fruit ripening is a complex process which seems to be largely regulated by plant hormones. In contrast to climacteric fruit, the ripening of non-climacteric fruit, e.g. strawberries and grapes, is less dependent on ethylene and is controlled by several other hormones. We are investigating the role of hormones during grape berry development and are testing their ability to manipulate ripening. The application of some hormones, e.g. abscisic acid and brassinosteroids, can advance the onset of berry ripening (veraison) others, e.g. auxins, delay it. Of particular interest is the ability of synthetic auxins to retard grape berry ripening and therefore harvest, which has potential advantages for wine producers facing the challenges of a changing climate. A decrease in levels of indole-3-acetic acid (IAA), the most abundant auxin in plants, occurs during the pre-veraison stage of berry development. The potential for IAA to act as an endogenous inhibitor of grape ripening is a current focus of our research. Ethylene can interact with the IAA biosynthesis pathway and depending on the timing of application either promotes or delays ripening, mediated by changes in IAA levels and metabolism. We have shown that a family of IAA-amido synthetases is likely to be key in controlling the levels of active IAA in developing grape berries. These enzymes join IAA to a range of amino acids forming conjugates that, depending on the amino acid substrate, can serve as storage forms of free IAA or destine the active auxin for degradation. We have recently elucidated the 3-D structure for one of the grape IAA-amido synthetases that provides insights at the molecular level into an important mechanism involved in auxin homeostasis.

SYM-01-03

APICAL DOMINANCE, THE POWERFUL ROLE OF SUGAR DEMAND AND THE SECOND FIDDLE ROLE OF HORMONES

Beveridge C.A.1, Mason M.G.1, Ross J.J.2, Babst B.A.3, Kerr S.1, Gui R.4 and Weinclaw B.2
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Growing shoot tips can maintain dominance over the growth of axillary buds in a process termed apical dominance. This is typically thought to be under the control of the basipetal flow of auxin from the shoot tip. Here we show that the shoot tip, which is also a hungry consumer of mobile sugars, acts to prevent the initial growth of axillary buds by affecting the levels of sugars available for the buds. Mobile photobiosimmulates/sugars move at 150 cm per hour in tall garden pea plants. After decapitation, photobiosimmulate supply to the lower stem is enhanced at a rate of at least 30 cm per hour and correlates with the timing of bud outgrowth. Exogenous sucrose can induce bud release in intact plants in a dynamic response similar to decapitated plants. Sucrose induces the same transcription factor in buds previously reported to be regulated by plant hormones to control bud outgrowth (BRCT, BRANCHED1). In contrast, auxin moves at about 1 cm per hour and too slowly for auxin depletion after decapitation to induce the early growth of axillary buds. Treatments that reduce auxin content do not lead to bud release unless sugars are also increased by these treatments. Sucrose is therefore both necessary and sufficient to induce bud release. Auxin treatment to the cut surface of decapitated shoots does not prevent the early growth of buds and an auxin transport inhibitor supplied directly to axillary buds of decapitated plants also fails to prevent early bud outgrowth. This discovery establishes a paradigm for apical dominance, whereby sugar supply, controlled by shoot-tip demand, has an important regulatory role in the initial release of buds for growth and that the plant hormones, auxin, strigolactone and cytokinin gain in importance once the release has occurred. These findings published in PNAS will be updated with recent work on auxins, cytokinins and strigolactones.

SYM-01-04

SPATIAL METABOLIC PROFILING OF THE BARLEY ROOT IN RESPONSE TO SALT STRESS

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Abiotic stresses are major causes of crop yield losses in agriculture significantly impacting on sustainability. Barley (Hordeum vulgare L.) is the most salt tolerant cereal crop and therefore is a good model to study salt tolerance mechanisms in cereals. Parents of genetic mapping populations of barley cv. Clipper and Sahara have previously been shown to have a contrasting root growth phenotype in response to the early phase (osmotic component) of salinity stress with Clipper maintaining a significantly higher relative root elongation rate [1]. In order to determine spatial changes in primary metabolites involved in osmotic adjustment in barley roots, we profiled primary metabolites (amino acids, sugars, fatty acids) in three different regions of the root; root cap/cell division zone, elongation zone and maturation zone. In response to growth on salt media significant differences in the accumulation of primary metabolites were observed in the different regions of the root tip, and between salt-tolerant and salt-sensitive genotypes. For example, the deposition of some amino acids, such as proline, increased in both genotypes towards the root tip, however an increase in the plant hormone, indole-3-acetic acid (IAA), was only observed in the root tip of the salt-tolerant genotype. These significant metabolic responses to salt-stress are currently being investigated. [1] Shelden MC et al. (2013) Funct. Plant Biol. 40(5):516-530.
**SYM-01-05**

**NEW FUNCTIONS FOR FLAVONOIDS IN LEGUME NODULE DEVELOPMENT**

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Flavonoids are a diverse class of secondary metabolites that play roles in pigmentation, as antioxidants, in the control of auxin transport and in communication with bacteria. We are interested in the different roles flavonoids play in the interaction of legumes with nitrogen-fixing bacteria, which leads to the development of root nodules. To this end we have modified the flavonoid pathway in the model legume, *Medicago truncatula*, using RNA interference to inhibit specific branches of the flavonoid biosynthesis pathway. This was coupled to microarray analysis to study changes in gene expression in the flavonoid-deficient plants. This approach revealed that flavonoids are necessary for successful nodule development as well as nodule infection, and that the major differences in gene expression between flavonoid-deficient and control roots involved defense and hormone responses. We then used LC-MS/MS and auxin transport assays to characterize the changes in auxin during nodule development. We showed that flavonoids, in particular flavonols and isoflavonoids, are required for correct regulation of polar auxin transport and auxin accumulation. Using a nodulation mutant, we demonstrated that the induction of specific flavonoids modulate auxin transport is under the control of cytokinin signaling through the cytokinin receptor, CRE1. This links the action of cytokinin with the control of auxin transport during nodule organogenesis.

**SYM-02-01**

**USING COMMUNITY AND ECOSYSTEM GENETICS TO DEVELOP GENETICS-BASED SOLUTIONS TO CLIMATE CHANGE AND OTHER GLOBAL CHALLENGES**

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As foundation species are by definition, community and ecosystem drivers, their genetic structure and extended phenotypes on the community and ecosystem, are especially important to understand and quantify. Our findings show that different tree genotypes support different communities of soil microbes, mycorrhizae, arthropods, vertebrates, understory plants, lichens, and pathogens, and that these differences can be quantified as heritable plant traits. Because different plant populations and individual genotypes vary predictably in the communities they support, and in their responses to climate change and invasive species, genetics-based approaches can provide important solutions to mitigate global change impacts on wild lands. Specifically, we are using an array of replicated common gardens along elevation and latitude gradients to quantify gene x environment interactions and to identify plant populations, specific genotypes, and key genetics-based interactions that can be deployed at restoration sites or used in assisted migration that will meet conservation goals in future climates. I will develop some of our findings using the newly funded Southwest Experimental Garden Array and describe how land managers in federal, state and private agencies are participating and utilizing our findings to mitigate the effects of climate change and invasive species. We seek global partners to further expand this approach and to identify broader generalities and applications that might be achieved.

**SYM-02-02**

**EUCALYPTUS LANDSCAPE GENOMICS**

Bragg J.G.

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How do we best choose seed material for re-vegetation, in the face of changing climate? For forest trees, these choices are likely to be very important, since their responses to environmental change will have substantial consequences for ecosystem function. I’ll describe a project, recently initiated with a large group of collaborators, that aims to describe links between genotypes, phenotypes, and the environment in two eucalypt species. I will discuss our sampling strategy, and results of some preliminary analyses.

**SYM-02-03**

**EVOLUTION OF A ICONIC WIDE SPREAD EUCALYPT, THE RIVER RED GUM, IN RESPONSE TO CLIMATE CHALLENGES**

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Widespread Eucalyptus species traverse strong environmental gradients, providing excellent opportunities to study genomic adaptation to climate. *Eucalyptus camaldulensis* (river red gum) occurs in riverine and floodplain habitats Australia-wide. River red gums depend on flooding for recruitment, but adults can withstand prolonged periods of drought, suggesting evolved mechanisms to cope with extremes in water supply. We are examining hydrologically contrasted populations of river red gum at different spatial scales to better understand how such adaptations have influenced genetic diversity in this species. To do this we have sampled more than 1000 trees capturing age and altitudinal classes that differ in flooding history within populations (local scale), at 12 sites along the Murray River (regional scale). We have also sampled 370 individuals from 37 climatically diverse sites reflecting the species distribution Australia-wide (continental scale). A set of 700 single nucleotide polymorphism (SNP) loci identified from whole genome and amplicon sequencing have been genotyped across these populations, and the data applied to examine evidence of genetic adaptation in relation to flooding history and local climate at varying spatial scales. Specifically we address whether adaptation to water availability has resulted in genetic differentiation among populations, and examine whether adaptive signals change when compared over different evolutionary time scales. In addition we are establishing phenotype-genotype links via association studies for a subset of these loci which is providing insight into how selection is acting by exposing which phenotypes are responsible for adaptive patterns. These findings contribute to a greater understanding of the adaptive potential of eucalypt populations threatened by changing climate, and can be directly applied to inform forest management and conservation.
SYM-02-04
GLOBAL PATTERNS OF HEAT-WAVE RISKS TO HIGHER PLANTS
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3Hawkesbury Institute for the Environment, University of Western Sydney, Penrith, NSW.

Thermal tolerance in plants is of increasing importance in a warming world. In this presentation, we report on a study of 218 species at 19 sites in the first to assess global patterns of high-temperature tolerance of plant energetic metabolism. We found that thermal tolerance declined from high to low latitude (with ~8°C range when comparing site means). This study also found large differences in thermal safety margins and thus the greatest risk of damage due to extreme heatwave events. We also found that there are large differences in thermal tolerance among co-occurring species at each site, and a significant number of species in most biomes will be at risk in the future as heatwave events become more severe due to climate change.

SYM-02-05
IDENTIFYING GENETIC ARCHITECTURE OF ADAPTIVE TRAITS IN BRACHYPODIUM IN VARIABLE CLIMATES
CoE Plant Energy Biology, Research School of Biology, The Australian National University.

Food security is a major issue around the world with global crop production needing to nearly double by 2050, meanwhile using inputs more efficiently and dealing with increasing climate variability. Our research aims to examine the three way relationship between genotype, phenotype and environment, to directly identify and then pre-select genetic loci providing crop species with high yield potential and stability in changing climates. We are validating this approach using the new model grass Brachypodium, which is closely related to wheat and barley. We have gathered over 1500 natural accessions from a wide range of climates and locations, compiling existing collections and new accessions from Australia. Genomic sequencing has identified ploidy variation, population structure and 100,000s of SNPs for use in Genome Wide Association Studies (GWAS) to find causative alleles for adaptive traits. We are using climatron growth chambers which mimic modelled regional field conditions in the past, present or future with diurnal and seasonal cycles in temperature, humidity, light intensity and light spectrum. On top of climate variation we are imposing abiotic stress including water, light and temperature extremes. Growth, development, and yield phenotypes are measured using images taken from in-chamber cameras, using the Trayscan system (PSI) as well as manual measurements. Preliminary experiments have focused on traits theorised to be important for yield in Australian wheat. Timing of flowering is extremely important to avoid sterility due to frosts and reduced grain filling due to terminal droughts. Traits affecting biomass accumulation have also been measured including early vigour, photosynthetic efficiency and yield components. Results thus far have revealed putative causative alleles for these traits, many of which may be novel. Future experiments will confirm these alleles and examine the effect of climate change on these traits and their genetic basis.

SYM-03-01
IDENTIFICATION OF HEMOGENIC PRECURSOR CELLS IN VIVO
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Definitive hematopoiesis emerges during embryogenesis via an endothelial-to-hematopoietic transition in the aorta-gonad-mesonephros (AGM) region and placenta. This developmental process remains poorly understood mainly due to the lack of specific markers for the prospective isolation of definitive hemogenic precursor cells. We have recently demonstrated the induction of hematopoietic stem/progenitor cells (hHSPCs) from fibroblasts with transcription factors (Pereira et al Cell Stem Cell, 2014). Guided by our programming experiments we analysed midgestation mouse placentas for a putative hemogenic precursor cell phenotype. We identified a small population of CD34+Sca1+Prom1+ (34SP) cells that do not express the pan-hematopoietic marker CD45. After culture 34SP cells acquire the expression of CD45 and generate large hematopoietic and cobblestone-like colonies. 34SP cells express markers associated with hemogenic endothelium and are heterogeneous for early hematopoietic commitment markers. Remarkably, global gene expression profiles of placental 34SP cells correlate with AGM-derived hemogenic endothelium and our fibroblast-derived precursors. Integration of this data generated a stringent gene expression signature for definitive hemogenic cells. Finally, when co-cultured with stroma 34SP cells give rise to B, T cells and multi-lineage myeloid colonies. Most importantly, these cells also repopulate mice. In summary, we show that direct in vitro conversion provided a cell surface phenotype for the isolation of hemogenic precursors in vivo. Our findings provide insights into the specification of definitive hemogenesis in the placenta, in depth characterization of hemogenic precursor populations and the first evidence that direct in vitro conversion approaches can be used as a valuable tool to address basic developmental questions.

SYM-03-02
DISSECTING PATHWAYS OF HUMAN HEMATOPOIETIC DEVELOPMENT USING PLURIPOTENT STEM CELLS
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The capacity to grow and differentiate human pluripotent stem cells has greatly impacted on our understanding of normal development and disease, provided tools for the pharmaceutical and biotech industries and now represents a source of cells for a potentially broad range of clinical applications. Research in our laboratory encompasses the generation of mesodermal and endodermal lineages from human pluripotent stem cells (hPSC). Our underlying strategy is to guide hPSC differentiation along the same developmental pathways traversed by cells during the ontogeny of these cell types during embryogenesis. To facilitate lineage analysis, we have genetically modified hPSCs by inserting DNA sequences encoding fluorescent protein reporters into gene loci whose expression marks a spectrum of developmental stages from undifferentiated hPSCs to mesendodermal precursors to cells of more differentiated lineages. We are using these tagged cell lines to refine the growth factor requirements for directed differentiation of hPSCs, to identify new cell surface markers and to generate, isolate and characterise purified populations of viable cells. I will focus on our recent studies in which we have been using reporter lines for RUNX1C and SOX17 to assist in dissecting the emergence and development of hematopoietic cells from hemogenic endothelium.
**SYM-03-03**

GENETIC FATE MAPPING OF MESENCHYMAL STEM-LIKE CELLS AND THEIR CONTRIBUTION TO DEVELOPMENTAL HEMATOPOIESIS

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Despite advances in our understanding of how hematopoietic stem cells (HSCs) are formed in the embryo, translating this knowledge into novel protocols for in vitro production of HSCs has proved difficult. Unlocking the role of accessory cells at sites of HSC production in the embryo will help clarify whether they constitute a missing element in current protocols. The earliest HSCs originate from a population of haemogenic-endothelial cells that line the ventral surface of the dorsal aorta in the aorta-gonad-mesonephros (AGM) region of the E10.5 embryo. In this tissue, at the time of HSC emergence, we have identified existence of mesenchymal stem-like cells (MS-LCs) marked by a surface receptor Pdgfra and an intermediate filament Nestin (N-GFP). These MS-LCs showed long-term self-renewal and multipotency of mesodermal and ectodermal lineages but not endodermal lineages. Pdgfra+/N-GFP+/CD45−/MS-LCs possess both CFU-C (blood) and limited CFU-F capacity, whereas Pdgfra+/N-GFP−/CD45−/MS-LCs showed high level of CFU-F capacity with vascular lineage specificity in vivo. Conditional ablation of Nestin+/Pdgfra+ or Nestin−/Pdgfra+ MS-LCs in the AGM led to either partial or complete loss of MSC activity, severe loss of both endothelial and pericyte-like cells and concomitant loss of blood formation. Inhibition of Pdgfra signalling in the AGM led to reduction in CFU-C; CFU-F and pericyte activity in a dose dependent manner. Lineage tracing studies showed that these MS-LCs originated from the mesoderm (Mesp1) and neural crest (Wnt1) but not from the neuro-epithelium (Sox7). Taken together we describe a novel population of MS-LCs and their contribution to blood formation in the AGM.

**SYM-03-04**

ANALYSIS OF HUMAN GLOBIN GENE SWITCHING DURING EMBRYONIC STEM CELL DIFFERENTIATION

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Over 7% of the global population are carriers of haemoglobinopathies. However, despite their prevalence, no effective treatments exist for ß-thalassemia and sickle-cell anaemia beyond lifelong blood transfusions. The globin genes are expressed sequentially during development, thus different compositions of haemoglobin exist at the embryonic, fetal and adult stages. Individuals with large deletions encompassing the adult ß-globin gene exhibit compensatory expression of fetal γ-globin into adulthood, resulting in the benign condition hereditary persistence of fetal haemoglobin. The amelioration of the symptoms of β-globinopathies in the rare cases where these disorders are co-inherited has inspired decades of research into the molecular regulation of haemoglobin switching, with the aim of inducing therapeutic γ-globin expression. In spite of this, the mechanism remains unsolved, partly due to the lack of suitable cell and animal models available. Our lab has developed a novel system for studying and manipulating haemoglobin switching in vitro. We have generated mouse embryonic stem cell lines harbouring a 200kb bacterial artificial chromosome (BAC) containing the full human ß-globin locus, including its upstream regulatory regions. The BAC contains GFP and DsRed reporter constructs under control of the ß-globin and γ-globin promoters, respectively (Chan, et al. 2012, The FASEB Journal). During hematopoietic differentiation of our embryonic stem cell lines, we have shown sequential expression of the DsRed and GFP reporter genes by fluorescence microscopy and flow cytometry, confirming that our system recapitulates haemoglobin switching during ontogeny. Using this system, we aim to screen chemical and genetic modifiers of γ-globin expression to identify new treatment avenues for β-globinopathies.

**SYM-03-05**

MIR-155 REDUCES COLONY FORMATION AND PROLIFERATION OF AML IN VITRO, BUT DOES IT DELAY DISEASE ONSET IN VIVO?

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1Walter and Eliza Hall Institute (Melbourne). 2University of Melbourne (Melbourne). 3Centre for Cancer Biology (Adelaide).

MicroRNAs are a class of small, non-coding RNA species that post-transcriptionally repress their mRNA targets. The regulation of cell fate by microRNAs includes regulating survival, proliferation and differentiation. These functions implicate microRNAs in developmental processes such as hematopoiesis and microRNA expression is often dysregulated in hematological malignancies and other cancers. In a model of induced expression of an oncogene in primary murine myeloid cells, we identified approximately 70 microRNAs that were differentially expressed in the presence and absence of the oncogene. This raised the hypothesis that at least some of these microRNAs have roles in the regulation of myeloid differentiation and in the development of myeloid leukemia. One of these differentially expressed microRNAs, miR-155, is the focus of the work presented here. MiR-155 has been previously published as an oncogenic microRNA in lymphoid and other cancers. In contrast, our expression profiling data of primary murine acute myeloid leukemia (AML) models demonstrated a reduction in miR-155 expression in AML cells compared to non-malignant controls, suggesting a potential role for miR-155 as a tumour suppressor. This was further supported by in vitro competition assays and clonogenic assays in ex-vivo murine AML cell lines, where overexpression of miR-155 resulted in a reduction in the rate of proliferation and colony formation. The effect of miR-155 overexpression on engraftment and development of AML in vivo, will also be presented. These experiments will establish if miR-155 delays AML onset and progression, in vivo.

**SYM-04-01**

THERAPEUTIC ALTERNATIVE SPLICING: OPPORTUNITIES AND CHALLENGES

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Splicing is a fundamental process during the expression of most human gene transcripts, with alternative splicing frequently occurring in a tissue-specific and/or developmental manner to further increase our genetic plasticity. We have shown that antisense oligomers can specifically redirect pre-mRNA processing by either exciting a selected exon (blocking positive enhancer elements), or promote retention of an exon normally excluded from the mature mRNA (masking splice silencer motifs). Therapeutic alternative splicing is now in clinical trials to address Duchenne muscular dystrophy (DMD), the most common and serious form of childhood muscle wasting. Protein truncating mutations in the DMD gene that preclude synthesis of a functional protein can be removed during dystrophin pre-mRNA processing with splice switching oligomers. Targeting dystrophin exon 51 for excision will restore functional dystrophin expression in the most common subset of DMD deletion patients (about 10% of DMD boys). Following Phase 1 and 2a trials in the UK, an extended placebo-controlled study was initiated under the sponsorship of Sarepta Therapeutics in Nationwide Children’s Hospital, Columbus, Ohio. The trial has now been underway for nearly 3 years and clinically significant differences were observed, with treated boys maintaining similar levels of ambulation over the trial period. No serious adverse events have been reported and the trial remains ongoing. These promising DMD trial results have rekindled enthusiasm to pursue splice intervention therapies for other disorders. Spinal muscular atrophy, cystic fibrosis, myotonic dystrophy, facioscapulohumeral muscular dystrophy, asthma, Alzheimer’s, Parkinson’s and stroke are just some of the conditions currently under investigation in our laboratory. An estimated 15% of human mutations induce aberrant splicing and splice switching oligomers may be used as a personalized genetic therapy, regardless of the mutated gene.
SYM-04-02
IDENTIFICATION OF 2500 NOVEL PROTEIN ISOFORMS ARISING FROM AN UNUSUAL OUTCOME OF ALTERNATIVE SPLICING
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Fahrer A.M. and Wilson L.O.W. Research School of Biology, the Australian National University.

We have studied an unusual outcome of alternative splicing, which results in the amino terminus of a protein being translated from alternate reading frames. In each case, the alternatively spliced mRNA differs from the canonical mRNA by a length of sequence not divisible by 3, however translation can be initiated at an alternate start codon; rescuing the reading frame of the 3′ mRNA sequence (downstream of the alternative splicing), but changing the upstream reading frame. This results in predicted protein isoforms with completely novel amino-end sequences. A bioinformatics approach was developed to identify these proteins, by analyzing public EST databases. 3045 such isoforms were identified from 7 different species (mouse, human, rat, Arabidopsis, C. elegans, Drosophila and zebrafish). Of these, 2567 (84%) are novel. Evidence supporting the translation of a selection of mouse and human proteins was obtained from mass spectrometry analysis, and from a published study of ribosome initiation sites. As automated genome annotation pipelines do not routinely consider alternative start codons, the majority of these protein isoforms have never previously been identified. Only one instance has previously been studied in detail, and converted the encoded protein (OTUB1) from an activator of T cell-function to a suppressor, demonstrating that these protein isoforms can result in profound functional change. The variants we have identified are involved in a wide variety of biological processes, and therefore open up many new areas of research. We have published the mouse and human sequences, but not yet those from the other genomes, and have begun a new collaborative approach in working with Arabidopsis, C. elegans, Drosophila or zebrafish.

SYM-04-04
ATRX DEPENDENT DEPOSITION OF H3.3 AT HETEROCHROMATIN IS NECESSARY FOR THE MAINTENANCE OF CHROMATIN STATES
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Histone H3.3 is a replication-independent histone variant which replaces histones displaced outside S-phase. H3.3 deposition at euchromatin is dependent on HIRA while ATRX/Daxx are necessary for H3.3 deposition at pericentric heterochromatin and telomeres. The role of H3.3 at heterochromatic regions is currently unknown but mutations in the ATRX/Daxx/H3.3 pathway have been linked to aberrant telomere lengthening in certain cancers. In this study, we show that ATRX-dependent deposition of H3.3 is not limited to pericentric heterochromatin and telomeres but occurs at a number of heterochromatic sites throughout the genome. Notably, ATRX/H3.3 was found to localise specifically to the DNA methylated allele at most imprinted DMRs in mouse ES cells. ATRX KO cells failed to deposit H3.3 at these sites leading to loss of the H3K9me3 heterochromatin modification and aberrant allelic expression. We propose a model whereby ATRX-dependent deposition of H3.3 into heterochromatin is required for maintaining silencing modifications at these sites.

SYM-04-03
EPIGENOME PROFILING OF PRIMARY HUMAN SUBCUTANEOUS AND VISCERAL ADIPOCYTES REVEALS DIFFERENCES IN POTENTIAL REGULATION OF DEVELOPMENTAL AND METABOLISM GENES
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1CSIRO, Food and Nutrition, Sydney. 2The Garvan Institute of Medical Research, Sydney. 3St Vincents Hospital, Sydney.

Adipose tissue plays an important role in metabolism and homeostasis. Perturbations in the nature and amount of adipose tissue have serious health consequences for individuals and society. An increase in visceral adipose tissue (VAT) is associated with diseases ranging from Type 2 Diabetes Mellitus to cancer, while subcutaneous adipose tissue (SAT) may have a protective effect. Numerous genes have been identified to be differentially expressed between these two adipose depots, however mechanisms controlling this differential expression is poorly understood. To characterize potential developmental differences in adipocytes from different depots we analysed both the transcriptomes and methylomes of adipocyte cells purified from SAT and VAT of three live females. Methylomes were mapped using both whole genome bisulphite sequencing and illumina 450k arrays and gene expression characterized using Affymetrix Gene 2.0 arrays and strand-specific RNA-Seq. We found that methylation profiles of each sample type were highly consistent between patients, clearly segregating cell types. While distinct DNA methylation differences were found between purified visceral adipocytes and whole visceral adipose tissues, they clustered closely and separated clearly from subcutaneous adipocytes. The observed methylation differences indicate the separate developmental origins of subcutaneous and visceral adipose cells, while differences in expression of lipid metabolism and inflammatory response genes may reflect functional and environmental differences respectively between the two tissues. The primary epigenomic profiles of purified normal adipocytes form the basis for understanding the functional role of different fat depots in metabolic disease.

SYM-04-05
CREATING AND CHARACTERIZING EPIALLELES IN WHEAT

DNA methylation is a well characterized epigenetic mark that plays an important role in many biological processes including the silencing of transposable elements, regulation of imprinted gene expression, control of recombination and the organization and stability of the genome. In Arabidopsis, genome-wide loss of DNA methylation due to reduced expression of MET1, the major DNA methyltransferase, has severe consequences on plant development. In contrast, local changes in DNA methylation can lead to phenotypic variation associated with the formation of epialleles, allelic variants that show altered gene expression associated with changes in DNA methylation. A population of Arabidopsis EpiRILs, recombinant inbred lines differing only in the location of DNA methylation, shows heritable variation in many traits. Naturally occurring epialleles in toadflax, rice and maize confer heritable phenotypic variation over generations. This raises the questions of whether plant breeders have inadvertently selected variation due to epialleles for crop improvement, and whether manipulating DNA methylation can be used as a tool to enhance phenotypic variation of agronomic traits. We have used chemical disruption of DNA methylation in wheat to explore the possibility of creating novel epigenetic variants for crop improvement. Plants with heritable changes in flowering time, dormancy and spike architecture have been identified and we are currently characterizing the molecular basis for these phenotypes.

Page 30  ComBio2014 • Canberra, ACT, Australia • 28 September – 2 October, 2014
SYM-05-01
THE TWO FACES OF HIPPO: THE HIPPO SIGNALING PATHWAY IN MAMMALIAN DEVELOPMENT, REGENERATION AND DISEASE

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The Hippo signaling pathway is an emerging growth control and tumor suppressor pathway that regulates cell proliferation and stem cell functions. Defects in Hippo signaling and hyperactivation of its downstream effectors YAP and TAZ contribute to the development of many diverse solid tumors. These observations have led to the suggestion that inhibition of YAP and TAZ activity may be a novel means to aid in the treatment and/or prevention of cancers that exhibit deregulated Hippo signaling. In contrast to their oncogenic roles, YAP and TAZ can also play beneficial roles in stimulating tissue repair and regeneration following injury. Thus, activation of YAP and TAZ may be useful in situations where transient stimulation of cell proliferation and survival would aid repair and regeneration of tissues. In our laboratory, we are taking a genetic approach to identify essential roles for Hippo signaling in mammalian development, homeostasis and disease. Recent ongoing studies that highlight the role of Hippo signaling in development, tumor suppression, and regeneration will be discussed.

SYM-05-02
LGL REGULATES NOTCH SIGNALING VIA ENDOCYTOSIS IN THE DEVELOPING DROSOPHILA EYE

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The Drosophila melanogaster junctional neural tube tumor suppressor, Lethal-2-giant larvae (Lgl), is a regulator of apico-basal cell polarity and tissue growth. We have previously shown in the developing Drosophila eye epithelium that, without affecting cell polarity, depletion of Lgl results in ectopic cell proliferation and blockage of developmental cell death due to deregulation of the Hippo signaling pathway. Now we show that Notch signaling is increased in lgl depleted eye tissue, independently of the function of Lgl in apico-basal cell polarity. The upregulation of Notch signaling is ligand-dependent and correlates with accumulation of cleaved Notch. Concomitant with higher cleaved Notch levels, early endosomes (Avlanch), recycling endosomes (Rab11), early multivesicular bodies (Hrs) and acidified vesicles accumulate, but not later endosomal markers (Carnation and Rab7). Upregulation of Notch targets in lgl mutant tissue is independent of Hrs/Siam or Rab11 activity but requires vesicle acidification. Furthermore, Lgl regulates Notch signaling independently of the apical polarity complex. Altogether our data shows that Lgl restricts vesicle acidification to prevent ectopic Notch signaling. This function for Lgl uncovers a novel attenuation mechanism of ligand activated Notch signalling during Drosophila eye development.

SYM-05-03
DEFINING THE ROLE OF INNATE IMMUNITY AND NERVE SIGNALLING IN THE REGULATION OF ADULT SALAMANDER LIMB REGENERATION

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In response to injury, adult mammals possess a limited capacity to regenerate and restore complete function to damaged tissue and organs. In contrast, adult salamanders display a remarkable capability to resolve injury, and can regenerate complex structures such as amputated limbs or damaged hearts, brains and spinal cords. Regeneration of a new limb is accomplished through the formation of a progenitor pool that forms within the presence of damaged nerve. The progenitor pool that forms within this wound then undergoes differentiation and patterning into a new limb. Using the axolotl (Ambystoma mexicanum) as a model to study vertebrate regeneration, we have previously demonstrated a temporally defined requirement for innate immune cells such as macrophages during the limb regeneration process and identified nerve dependent signals capable of rescuing regeneration in the absence of nerve. We have established a FACS based protocol and tool kit to isolate specific cell populations during the early phase of axolotl limb regeneration and have performed gene expression analysis to identify candidate molecules involved in the regeneration specific response. These studies provide the foundation to identify and investigate novel genetic programs that promote scar-free wound healing or regeneration and define important aspects of the neuro-immunological axis of regeneration. By understanding scar-free healing and regeneration in amphians it is hoped that we can improve repair outcomes in adult human tissues.

SYM-05-04
POLARITY PROTEIN CONTROL OF EPITHELIAL PLASTICITY

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The defining feature of epithelial cells is the development of specialised cell surface domains, defined by their unique protein and lipid components. The apical domain of epithelial cells comprises the luminal surface in branching epithelial organs. The cellular polarity machinery supports domain development via a network of mutually antagonist protein-protein interactions. Without appropriate cellular polarity epithelial tissue is dysfunctional, and loss of polarity is evident in cancer. The Lethal giant larvae (Lgl) complex is a core component of the cellular polarity machinery, acting to define basolateral membrane identity by limiting apical domain expansion. Notably, loss of Lgl contributes to cancer progression, but the molecular mechanisms of Lgl function in lumogenesis and disease progression are poorly understood. Lgl interacts with components of the exocytic and actomyosin machinery, yet it is unclear if the morphologic consequences of Lgl dysregulation are due to defects in trafficking or cortical organisation, or both. We aim to define the molecular mechanism/s and signalling pathways that contribute to Lgl function in development and disease. Depletion of Lgl caused overgrowth of cells into the lumen using a 3-dimensional organotypic culture system; akin to overgrowth observed in mutant Drosophila tissues and the brains of Lgl -/- mice. Loosening of cell polarity enhanced migratory and invasive cell behaviours, and caused dysregulation of the JAK/STAT and Notch signalling pathways, changes that are associated with resistance to apoptosis, and enhanced cell survival associated with human disease. This study highlights the importance of cellular polarity in maintaining normal epithelial function, and reveals the contribution of Lgl to regulation of oncogenic signalling pathways known to be involved in cancer progression.
SYM-05-05
TROPOMYOSIN BASED REGULATION OF ACTIN DYNAMICS AT THE LEADING EDGE OF MIGRATING CELLS

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One of the hallmarks of cancer is the development of metastases through the uncontrolled migration of cells away from the primary tumor to distant sites. This is achieved through dynamic remoulding of the actin cytoskeleton. By examining the regulatory mechanisms underlying cell migration, greater insight into cancer metastasis and disease progression can be obtained. The actin cytoskeleton is tightly regulated by tropomyosin, which exist in 40 isoforms of which exist in mammals. Furthermore, different isoforms sort to different cellular compartments, indicating isoform specific regulatory roles. Although motility is a widely studied cellular process, the role of tropomyosin in its regulation remains unclear. The principal step in cell migration involves actin-mediated protrusion of the leading edge plasma membrane to form lamellipodia. While much of the literature reports this region to be free of tropomyosin, we have demonstrated, using isoform specific antibodies, that two isoforms; Tm5a/5b are enriched in the lamellipodia of mouse fibroblasts, indicating a probable role in the regulation of actin filament function within this structure. Using live-cell imaging, kymography and cell tracking we have shown that knockdown of Tm5a/5b expression through RNA interference leads to lamellipodia which display a marked decrease in protrusion persistence with a corresponding reduction in whole cell motility and a reduction in the number of focal adhesions. These findings suggest that actin assembly at the leading edge is tightly regulated by Tm5a/5b which acts to stabilise filaments to assist in the propagation and maintenance of lamellipodia, enabling the cell to form adhesions to the substratum before generating contractile force. In this way, Tm5a/5b behaves as a spatio-temporal regulator of the cell migration cycle.

SYM-06-02
NOVEL ROLES FOR THE PSEUDOKINASES SGK223 AND SGK269 IN CANCER PROGRESSION

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The human genome encodes 518 protein kinases and pseudokinases, collectively referred to as the ‘kinome’. This enzyme superfamily harbours considerable untapped potential in terms of cancer drug targets and biomarkers. In addition, it is evident that many kinases implicated in cancer development remain poorly characterized. To bridge this knowledge gap, we are using an integrated strategy that combines mass spectrometry (MS)-based phosphoproteomic and kinomic profiling with functional genomics. This provides the analytical power to identify oncogenic kinases that contribute functionally to the ‘kinome’ and identify kinases that act downstream of the identified kinase network. We have used phosphoproteomic profiling to determine that the pseudokinase Sgk269/PEAK1 is overexpressed in triple-negative breast cancer and is a novel target of the Src family kinase Lyn. Sgk269 functions as a scaffold, playing a key role in temporal and negative breast cancer and is a novel target of the Src family kinase network.

SYM-06-03
RESISTANCE TO MAPK INHIBITORS IN MELANOMA: INSIGHTS FOR FUTURE THERAPIES

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The selective inhibition of the mitogen activated protein kinase (MAPK) pathway with combined BRAF and MEK inhibitors was recently approved by the Australian Therapeutic Goods Administration for the treatment of BRAF-mutant metastatic melanoma. Despite significant improvements in clinical outcomes and reports of long-term response, 50% of patients treated with combination MAPK inhibitors progress within one year and resistance remains a barrier to better patient outcomes. We examined the genetic profile of resistance mechanisms and tumour signalling pathway activity in 70 melanomas derived from 40 BRAF mutant melanoma patients receiving MAPK inhibitors. The relative frequency of these resistance mechanisms and their association with clinically relevant features and therapeutic outcomes was analysed. Defining the mechanisms of acquired resistance to MAPK inhibitors and their sensitivity to alternative second-line therapies may provide an opportunity for therapeutic exploitation, particularly in patients who show minimal clinical benefit.
The bromodomain protein, BRD4, has been identified recently as a therapeutic target in a variety of cancers and inflammatory disease. BRD4 has been shown to have pleiotropic functions. During interphase of the cell cycle, BRD4 binds to acetylated histones in chromatin through its bromodomains and recruits a variety of chromatin modifiers and transcription factors to enhancers and promoters. BRD4 also functions as a mitotic bookmark for genes expressed early in G1. Despite its role in a broad range of biological processes, the precise molecular mechanisms of BRD4 function have remained unknown. We report that BRD4 is a novel kinase that functions as a regulator of eukaryotic transcription. BRD4 binds to the carboxy-terminal domain (CTD) of RNA Polymerase II (Pol II) and directly phosphorylates its Ser2 sites, both in vitro and in vivo. Consistent with the requirement for Ser2 phosphorylation during early elongation, BRD4 is required for transcription. We suggest that BRD4 functions immediately after transcription initiation and Ser5 phosphorylation by TFIIH/CDK7 and before Ser2,5 phosphorylation by the elongation factor, PTEFb. Importantly, these three major CTD kinases - TFIIH/CDK7, BRD4 and PTEFb/CDK9 - engage in cross-talk, modulating their subsequent CTD phosphorylation to ensure an orderly and sequential progression from initiation to early elongation and to efficient elongation. Conditional deletion of BRD4 in the thymus abrogates normal thymocyte differentiation. Recent evidence mechanistically linking the functions of BRD4 as a mitotic bookmark and as a general transcription factor will be presented.

FLICKING THE SWITCH ON CANCER CELL SIGNALLING – TARGETING PROTEIN PHOSPHATASE 2A IN BREAST CANCER

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Background: Breast cancer is the most common cancer diagnosed in women, and despite recent advances in early detection and targeted therapies, it remains a leading cause of death worldwide. Breast cancer is characterised by disruption in cellular signalling pathways controlling proliferation, survival and migration. Protein Phosphatase 2A (PP2A), is a family of ser/thr phosphatases composed of a catalytic, a structural, and a regulatory subunit, of which there are multiple isoforms. PP2A complexes regulate numerous signalling pathways and are considered tumour suppressors, however a functional role for PP2A in breast cancer has not been described. Aims: To investigate the role of PP2A in breast cancer and determine its potential as a therapeutic target. Methods: PP2A gene (qPCR) and protein (immunoblotting) expression was examined in human breast cancer cell lines, and in primary tumours (n=39) by immunohistochemistry. Effects of PP2A knockdown (shRNA) was assessed in 3D cultures of MCF10A breast epithelial cells. Cytotoxicity and 3D culture assays were performed on MCF7 and MDA-MB-231 cells with pharmacological PP2A activators (FTY720 and AAL(S)). Results: We identified significantly reduced PP2A-A, PP2A-Bα and PP2A-B'α protein in human breast tumours and metastases, compared to matched normal mammary tissue. Reduced PP2A subunit protein, but not mRNA, was also observed in breast cancer cell lines. Functionally, knockdown of PP2A-Ba, -B' or B'y subunits induced increased cellular proliferation, resulting in enlarged, multilobular 3D acini, characterised by enhanced pAkt. Finally, FTY720 and AAL(S) inhibited proliferation of breast cancer cell lines, and displayed additive or synergistic cytotoxicity with standard breast cancer chemotherapeutics. Conclusions: This study provides clear evidence that PP2A inactivation is important in breast tumorigenesis, and suggests that targeting PP2A activation is a potential therapeutic strategy for breast cancer.
In this talk I shall seek to describe the early days of protein crystallography in the antipodes with reference to the people who made it all happen. I shall contrast the way experiments were carried out forty years ago compared with the modern methods and facilities we have access to today.

Rheumatoid arthritis (RA) is a chronic and debilitating autoimmune disease, characterized by inflammation of synovial tissue, joint pannus and bone erosion. RA has a strong association with a region of the HLA-DRB1 locus known as the ‘shared epitope’ (SE) and the presence of autoantibodies specific for citrullinated proteins. Previous studies have shown that citrullination of self-antigens can significantly increase the affinity of epitopes for SE alleles. Here we provide a molecular basis for how citrullinated vimentin and aggrecan epitopes can be presented by the SE alleles, HLA-DRB1*0401 and HLA-DRB1*0404. Citrulline was accommodated in the electropositive P4 pocket of HLA-DRB1*0401/04, whilst arginine was not. In addition, the RA resistant HLA-DRB1*0402 allomorph was capable of binding both arginine and citrulline in its electronegative P4 pocket. Peptide elution studies revealed that arginine was presented by HLA-DRB1*0402 but not by HLA-DRB1*0401/04. Moreover, citrullinated vimentin showed a greater sensitivity to proteolysis by cathepsin L, when compared to unmodified vimentin, indicating that citrullination can impact the repertoire of self-antigens presented. Using HLA Class II tetramers, we identified citrullinated vimentin and aggrecan specific CD4+ T cells from both HLA-DRB1*0401+ RA patients and healthy controls. In RA patients, the number of autoreactive T cells correlated with disease activity and were deficient in regulatory T cells compared to healthy controls. In RA patients, citrullinated vimentin and aggrecan specific CD4+ T cells from both HLA-DRB1*0401+ RA patients and healthy controls. In RA patients, citrullinated vimentin and aggrecan specific CD4+ T cells from both HLA-DRB1*0401+ RA patients and healthy controls. In RA patients, citrullinated vimentin and aggrecan specific CD4+ T cells from both HLA-DRB1*0401+ RA patients and healthy controls. In RA patients, citrullinated vimentin and aggrecan specific CD4+ T cells from both HLA-DRB1*0401+ RA patients and healthy controls. In RA patients, citrullinated vimentin and aggrecan specific CD4+ T cells from both HLA-DRB1*0401+ RA patients and healthy controls.
SYM-09-01

PLASMODESMATA: PORTALS CONTROLLING DELIVERY OF NUTRIENTS AND SIGNALING AGENTS

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Plant growth and development is controlled by a hierarchy of signals, driven by genetic, epigenetic, physiological and environmental inputs. At the cellular/tissue level, these inputs involve both cell-autonomously and non-cell-autonomously acting agents, including hormones, transcription factors (TFs), RNA - both large and small, proteins and peptides. In plants, positional information is transmitted by three main components, namely the mechanical/physical forces inherent to the developing tissue/organ, cell-cell communication by means of diffusible signals that cross the cell wall (apoplastic) barrier (hormones and peptide hormones), and cell-to-cell signaling through the symplasmic pathway established by plasmodesmata (PD) (proteins/TFs, mRNA, small RNA). 

Recent advances in real-time imaging, functional analysis of specific proteins from feed-forward, these three components, contributes to driving organogenesis down a particular pathway. Hence, knowledge of the signals and the cellular mechanisms underlying the transmission of such positional information is essential to build a comprehensive understanding of plant growth and development and consequently, to prevent or modify them when required. This concept, (sugars, amino acids, ions, etc.) delivery has long been appreciated, and recent studies have provided insights into the underlying mechanism(s) controlling this pathway. A considerable body of evidence has now accumulated that enzymes in the import role played by PD in cell-to-cell trafficking of a broad range of proteins, including TFs, cell cycle regulators and signaling agents. In this presentation, we will highlight recent findings on the molecular components of PD that function to regulate the movement of nutrient and signaling agents within the various symplasmic domains of the plant. Finally, we will direct attention to future studies aimed at developing a comprehensive model for the supramolecular structure of PD.

SYM-09-03

NETWORKS REGULATING HORMONE-MEDIATED PLANT GROWTH

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We are investigating hormone-mediated growth in Arabidopsis seedlings using transgenomic techniques, in order to understand the networks and signalling pathways regulating these processes. Our approaches include chromatin immunoprecipitation sequencing (ChiP-Seq) of 200 transcription factors (TFs) under native expression, in vitro determination TF DNA binding motifs, time series transcriptome analyses by RNA-Seq, genome-wide association studies, large-scale protein-protein interaction studies and forward genetics. Our initial studies have focused on responses to the phytohormone ethylene (ET), which has important roles in growth, development and defense. ET signalling involves a cascade of transcriptional reprogramming starting at EIN3, the master regulatory TF, which in turn activates RAV/EDF family TFs. EDF family TFs are involved in diverse plant defensive and hormonal pathways, and are potential novel integrators of hormone signalling and RNA silencing. We have revealed contrasting roles and functions of these TFs through sequential ChIP-Seq studies coupled to time-series RNA-Seq. Together they target the majority of the ET-responsive transcriptome. Data also indicates that EDF family members may have both unique and conserved functions. Currently, we are expanding our study to incorporate crosstalk with and between other hormone signalling pathways, by conducting ChIP-Seq on their primary TFs and by time series hormone response transcriptome profiling. Experiments have focused on the hormones jasmonic acid, salicylic acid and brassinosteroid. Results will be presented demonstrating how diverse data sources can be integrated to discover regulators of specific cohorts of hormone responsive genes. Furthermore, I will present data demonstrating the high degree of connectivity between diverse hormonal pathways.

SYM-09-04

THE ARABIDOPSIS PHOSPHATE EXPORTER PHO1: 3 DISTINCT DOMAINS INVOLVED IN SUBCELLULAR LOCALIZATION, PHOSPHATE TRANSPORT AND PHOSPHATE DEFICIENCY RESPONSE

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The Arabidopsis PHO1 is the prototypical phosphate (Pi) exporter, with homologues present in fungi and animals. In plants, PHO1 is primarily involved in the loading of Pi into the root xylem, and is thus essential for Pi transfer from roots to shoots. Consequently, the pho1 mutant shows low shoot Pi content. PHO1 has also been implicated in Pi signalling, impacting the response of plants to Pi deficiency. While the role of PHO1 in Pi homeostasis is well described, the structural basis for its functions is unknown and the role of PHO1 homologues is less investigated. Yet, all PHO1-like proteins share the same structural organization. They are composed of a hydrophilic N-terminal SPX domain, followed by 4 α-helices and a hydrophobic C-terminal EXS domain. While SPX and EXS domains are found in several eukaryotic proteins, their role is essentially unknown, although the SPX domain is found in several proteins involved in various aspects of Pi homeostasis. The membrane topology of PHO1, in particular the EXS domain, was determined using a combination of redox-sensitive GFP and bi-molecular fluorescence complementation (BFC). Expression of PHO1 variants containing various combinations of domains in both tobacco transient assays and complementation of the Arabidopsis pho1 mutant revealed that the 4 α-helices and EXS domain are the minimal region essential for Pi export, but that the SPX domain plays an important role.

The Arabidopsis pho1 mutant resulted in improvement of shoot growth despite low shoot Pi content. This results uncover a new role for the EXS domain of PHO1 in modulating the response of plants to Pi deficiency and uncoupling shoot growth response from Pi content.
SYM-09-05
EPISSTATIC REGULATION OF Cis-CAROTENOID SIGNALING METABOLITES AFFECTS CHLOROPLAST DIFFERENTIATION, NUCLEAR GENE EXPRESSION AND PLANT DEVELOPMENT

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Carotenoids are essential for all photosynthetic organisms and promote animal health. In plants, carotenoid derivatives can act as signaling metabolites, including abscisic acid and strigolactones. The next frontier is to discover what regulates carotenoid biosynthesis, storage and degradation. Previously, we showed that carotenogenesis is regulated by a chromatin-modifying enzyme, SET DOMAIN GROUP 8 (SDG8), which maintains expression of the carotenoid isomerase (CRTISO) in actively dividing tissues that can confer epigenetic change. CRTISO is a key rate-limiting enzyme and loss-of-function leads to the accumulation of upstream cis-carotenoids and altered compositions of downstream photoprotective pigments. The function of cis-carotenoid metabolites remains somewhat elusive. Here we report how cis-carotenoids accumulate in ctiso mutant (ccr2) tissues in response to environmental change and perturb chloroplast development (leaf yellowing) thereby altering retrograde gene expression and plant development. A forward genetics screen identified a revertant ccr2 plant (rccr2) that shows normal greening of leaf tissues and a different cis-carotenoid profile. Next generation sequencing and genetic analysis confirmed that a mutation in the zeta-carotene isomerase (ZISO), which acts upstream in the carotenoid pathway, restores chloroplast development as well as retrograde gene expression in ccr2. We discuss the metabolic nature of this epistatic interaction, which governs the accumulation of specific cis-carotenoids when exposed to a changing environment. The implications for linking plastid differentiation to the expression of genes involved in chloroplast to nuclear communication and developmental adaptation will be highlighted in the context of epigenetic memory forming processes.

SYM-10-02
L1 RETROTRANSPOSITION AND GENOMIC Mosaicism IN MAMMALIAN DEVELOPMENT

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LINE-1 (L1) retrotransposons are a class of “mobile DNA” found in all mammals. L1 occupies 17% of the human genome. ~100 transposition-competent L1 copies in each individual continue to cause new L1 insertions in gametes and somatic cells, driving genome structural variation as well as sporadic disease. In this talk, I will present data suggesting that L1 is active during mammalian development, particularly embryonic and adult neurogenesis. Through a targeted L1 sequencing approach, and whole genome amplification from single cells, we have identified L1 somatic mosaicism in embryonic stem cells, hippocampal neurons and hepatocytes. Somatic L1 insertions in neurons are arguably the most interesting as these variants typically impact genes expressed during neurogenesis, as well as enhancer elements active in the neuronal lineage. These observations suggest somatic mosaicism generated by L1 is likely to affect gene networks active in the same biological contexts in which L1 is mobile.

SYM-10-01
PLURIPOTENCY AND BEYOND

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One major research interest in our laboratory is focused on understanding the regulation of stem cell fate decision processes. A second interest is the use of stem cell approaches to develop patient-specific models of inherited diseases. Both of these efforts currently involve the use of embryonic or induced pluripotent stem cells (ESC and iPSC, respectively). We have taken a broad, quantitative systems biology approach to unravel the regulatory mechanisms that are necessary for maintaining and reacquiring the self-renewing pluripotent state. Specifically, we utilize short hairpin (sh) RNA techniques to perform loss-of-function perturbations in mouse (m) and human (h) ESC. We have developed a genetic complementation strategy to effectively “replace” any gene-product with a version that can be controlled by a small molecule added to cell cultures. Using this approach, we have perturbed the expression of numerous key regulatory molecules as transcription factors, epigenetic regulators as well as components of signaling pathways. After perturbation, we monitors global molecular changes over time. These changes include: chromatin modifications, mRNA levels, microRNA levels and the nuclear proteome. These studies provide a “real time” view of biological information processing that occurs during and is responsible for a transition in ESC fate. An essential component of our studies is computational biology. This has allowed us to analyze and integrate the large amount of information that is acquired. Computational analyses have also facilitated the generation of models of how regulatory networks function during changing cell fates. We have gained numerous novel insights into ESC regulation. Several of these include: how the Esrb transcription factor controls pluripotency, genotoxic stress response mechanisms that regulate ESC and translational control as an under-appreciated aspect of cell fate regulation. In our second major research focus, we have utilized iPSC reprogramming to develop models of human genetic diseases.

SYM-10-03
DROSHA-MEDIATED MESSENGER RNA CLEAVAGE IS NECESSARY FOR STEM CELL FUNCTION

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St Vincent’s Institute of Medical Research.

The decision of whether a stem cell should self-renew or to differentiate (as well as which lineage) is dependent on signals that converge to activate or silence specific genes. The shaping of its transcriptional profile is what ultimately controls the identity and function of a cell. We have discovered that mRNA cleavage by the RNase III enzyme Drosha plays a critical role in controlling stem cell function. Drosha is best known, along with Dicer, as one of the two enzymes required for the biogenesis of microRNAs in animals. However, by comparing the impact of deleting Drosha and Dicer in mice, we found that it is the non-microRNA function of Drosha that is particularly important in stem cells. Drosha recognises and cleaves protein-coding mRNAs that contain secondary stem loop structures. We show that degradation of specific mRNA targets is necessary for the function of haematopoietic and neural stem cells. Thus, mRNA cleavage represents an important level of regulation that contributes to the shaping of the transcriptional profile of stem cells.
SYM-10-04
UNRAVELLING EPIGENETIC COMPLEXES ASSOCIATED WITH GTF2IRD1 TO UNDERSTAND THE COGNITIVE FEATURES OF WILLIAMS-BEUREN SYNDROME
Carmona-Mora P.1, Tomasetti F.1, Canales C.P.1, Arshawa A.2, Dottori M.1, Hardeman E.C.1 and Palmer S.J.1
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GTF2IRD1 lies within a region of chromosome 7 that is prone to copy number variations resulting in distinctive neurobehavioural abnormalities. Hemizygous deletions cause Williams-Beuren syndrome (WBS) and duplications cause WBS duplication syndrome. Data from patients with atypical deletions and knockout mice show GTF2IRD1 as responsible for the most prominent of the cognitive and behavioural features of WBS including mental retardation and hypersociability. To understand the molecular basis of these defects, we studied for the first time, the localization of expression of endogenous GTF2IRD1 in human ES cell-derived neurons to correlate it with specific neuronal types and differentiation stages. Analyses in other cell lines show GTF2IRD1 as a nuclear speckle protein, colocalising with markers of gene silencing complexes. To define functional relationships, we used yeast two-hybrid screenings to isolate novel interaction partners and validated them in mammalian cells. Most are involved in chromatin modification and transcriptional regulation. Several partners were found to occupy the same compartment as GTF2IRD1, and some are implicated in mental retardation. We demonstrated the presence of GTF2IRD1 in chromatin modifying complexes and identified direct associations with histone deacetylases, proposing links with involved in chromatin modification and transcriptional regulation. Several novel interaction partners and validated them in mammalian cells. Most are involved in chromatin modification and transcriptional regulation. Several partners were found to occupy the same compartment as GTF2IRD1, and some are implicated in mental retardation. We demonstrated the presence of GTF2IRD1 in chromatin modifying complexes and identified direct associations with histone deacetylases, proposing links with histone deacetylases, proposing links with involved in chromatin modification and transcriptional regulation. Several novel interaction partners and validated them in mammalian cells. Most are involved in chromatin modification and transcriptional regulation. Several partners were found to occupy the same compartment as GTF2IRD1, and some are implicated in mental retardation. We demonstrated the presence of GTF2IRD1 in chromatin modifying complexes and identified direct associations with histone deacetylases, proposing links with histone deacetylases, proposing links with.

SYM-10-05
MICRORNA AND MRNA PROCESSING VARIATIONS IN CARDIAC BIOLOGY
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Introduction: Cells can produce both microRNAs (miRNAs) and their mRNA 3’ untranslated region (3’UTR) targets as multiple processing variants. We aim to comprehensively document these processing variants in the heart and determine whether they are altered during cardiac hypertrophy. Methods: Transverse Aortic Constriction (TAC) was performed to induce cardiac hypertrophy in C57BL/6 mice. RNAs were extracted from sham-treated, pre-hypertrophic (2 days post-TAC), and hypertrophic (7 days post-TAC) hearts, then subjected to next generation sequencing of small RNAs and mRNA 3’ends. Results: There is a widespread occurrence of microRNA processing variations in the cardiomyocyte, which remained largely unchanged during hypertrophy. Our mRNA 3’end-sequencing data identified novel 3’UTRs for 215 hypertrophy-related genes. Notably, there was a significant global shift towards shorter 3’UTR variants during hypertrophy. Statistical analysis shortlisted 101 mRNAs with a pronounced change in the proportion of 3’UTR variants, 7 of which have been validated through qPCR. Polysome fractionation revealed that 3’UTR lengths affected translational efficiency of mRNAs. Conclusion: We present in-depth information on microRNA and mRNA processing variants in cardiomyocytes. Importantly, the global shift towards shorter mRNA 3’UTRs, together with our demonstration of associated changes in translational efficiency presents a new paradigm of increased protein synthesis during hypertrophy. As a next step, we are now using our datasets to build a systems-level understanding of miRNA-mediated regulation during normal and hypertrophic states of the heart, which will aid in the development of microRNA-based therapy for cardiac disease.

SYM-11-01
NOT DIFFERENT, JUST BETTER: THE ADAPTIVE EVOLUTION OF A GLYCOLYTIC ENZYME
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Although we have a good understanding of adaptation at the organismal level, there is paucity of data addressing how organisms adapt at the molecular level. We ask how enzymes evolve: that is, what is evolution trying to optimize? Our study builds on a bacterial evolution experiment in which twelve bacterial populations have evolved in a glucose-limited environment for over 50,000 generations. Each population has greater fitness benefit was different in each case. Biochemical and fitness data to assess the effect of the adaptive mutations. To examine whether the evolved pykF alleles confer a fitness benefit in and of themselves, we replaced the wild-type pykF gene in the ancestor with each of the evolved pykF genes. Comparative fitness assays (against the ancestor) demonstrate that, although each of the pykF genes conferred a fitness benefit, the magnitude of the fitness benefit was different in each case. Enzymatic analysis demonstrates that the evolved enzymes have surprisingly different characteristics, with respect to activity, substrate binding and allosteric regulation. In addition, thermal stability assays demonstrate both increased and decreased stability when compared to the wild-type. The crystal structures and small angle X-ray scattering profiles for the evolved pykF enzymes showed surprising similarities to one another and the wild-type, despite the variability in fitness and function. Comparing the B-factor profiles of our structures, however, hint at differences in protein dynamics. Although the long-term evolution experiment demonstrates a high degree of variation with respect to fitness and mutational patterns, our data suggest much less parallelism with respect to protein function. Moreover, our results point to protein dynamics as an important mode for adaptive evolution in proteins. In this case, evolution seems to be acting upon protein dynamics to optimise enzyme function.

SYM-11-02
THE LINK TO DEFINING THE ALLOSTERIC MECHANISM OF AN OLIGOMERIC BACTERIAL AND PLANT ENZYME
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Dihydロpropionate synthase (DHDPS) is an oligomeric enzyme that catalyses the rate-limiting step in the lysine biosynthesis pathway of bacteria and plants. The pathway is regulated by feedback inhibition of DHDPS through the allosteric binding of lysine, which is the end product of the pathway. Although this represents a classical feedback inhibition process, the molecular mechanism of DHDPS allosterism remains elusive. Previous structural studies of well characterised DHDPS enzymes from Escherichia coli and Vitis vinifera (i.e. grapevine) show that no major conformational changes accompany lysine binding, indicating that the common concerted and sequential models of enzyme allosterism may not hold true to DHDPS. However, we show using biochemical analyses that DHDPS from Trichum aestivum (i.e. bread wheat) is allosterically regulated by lysine-induced dissociation of the active oligomeric species. This represents a rare example of the morpheein allosteric model – also known as the dissociative concerted mechanism – first proposed by Jaffe in 2005. Interestingly, our studies suggest that DHDPS is regulated by morphine principles, but that the mechanism is different in bacterial and plant species. Moreover, the allosteric model appears to be a proto-dissociative concerted process for T. aestivum DHDPS, where the binding of lysine induces dissociation of active tetramers to inactive dimers. By contrast, the binding of lysine to DHDPS from the bacterium Vibrio cholerae appears to conform to an associated concerted mechanism, where lysine induces association of the active dimeric form to an inactive tetrameric state. This is the first case where opposing morphine principles have been observed for orthologues from bacteria and plants. Accordingly, this mechanism has been coined the Ligand-Induced dis/associatioN by Lysine (K) (LINK) model, which offers insight into the evolution of enzyme allosterism and quaternary structure.
New model for NB-LRR activation.

Effectors AvrL567 bind preferentially to active receptors and propose a active state equilibrium of L6. Furthermore, we found that the fungal are involved in intramolecular interactions and control the inactive to regions in the TIR, the NB and the extra LRR C-terminal domains that comparisons and structure-guided mutagenesis, we identified several on the dimerization of its signalling TIR domain. Through L allele containing the AvrL567 effector. Using a structure-function analysis LRR, which confers resistance to the flax rust fungus (Melampsora lini) immune receptor is a Toll/interleukin-1 receptor (TIR) containing NB-LRR family. Plant immunity is triggered by the specific recognition of pathogen effectors by NB-LRRs, which often culminates into a localised cell death at the pathogen infection site. Thus, the activity of NB-LRRs proteins has to be tightly regulated to avoid inappropriate defence activation and cell death in absence of pathogen. In the flax plant system, the L6 immune receptor is a Toll/interleukin-1 receptor (TIR) containing NB-LRR, which confers resistance to the flax rust fungus (Melampsora lini) containing the AvrL567 effector. Using a structure-function analysis approach, we previously demonstrated that L6 activation depends on the dimerization of its signalling TIR domain. Through L allele comparisons and structure-guided mutagenesis, we identified several regions in the TIR, the NB and the extra LRR C-terminal domains that are involved in intramolecular interactions and control the inactive to active state equilibrium of L6. Furthermore, we found that the fungal effector AvrL567 binds preferentially to active receptors and propose a new model for NB-LRR activation.

**SYM-11-04**

**THE PROMOTER SEARCH OF RNA POLYMERASE: THE IMPORTANCE OF PROTEIN FLEXIBILITY**

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The slow diffusion of large macromolecules within cells limits the speed at which intracellular reactions can occur. Various mechanisms are observed in biological systems to help alleviate this constraint. Here, we investigate the promoter search of E. coli RNA polymerase (RNAP), focusing on the role of the two flexibly attached C-terminal domains of the α subunits (αCTDs). We present a physically realistic, yet conceptually simple, quantitative model for the promoter search of RNAP, which succeeds in unifying and replicating results from several independent experimental investigations. Our model shows that the flexible attachment of two αCTDs to the RNAP accelerates the promoter search via two distinct mechanisms. First, they increase the effective reactive surface area of RNAP, resulting in a greater probability of conversion to the closed complex per collision with DNA. Second, the αCTDs provide the means for short-range one-dimensional diffusion of the polymerase along the DNA, enabling faster search times. The model provides a useful case study for discussing the broader role of flexibility and entropy in biomolecular recognition.

**SYM-11-05**

**IMPROVING THE WORKHORSE: METABOLIC ENGINEERING OF THE NITROGEN CYCLE IN E.COLI**


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Sustainable production of chemicals and pharmaceuticals using microbial cell factories has garnered increased support in recent years due to concerns about depletion of crude oil feedstocks and environmental damage from current production processes. We report here the metabolic engineering of E. coli cells to develop an enhanced biological system for in vivo overproduction of amines, using citrulline as a suitable example. Our strategy focused on modification and deregulation of the arginine biosynthesis pathway. Arginine biosynthesis in E. coli is catalyzed by a multi-enzyme pathway which converts glutamine to arginine via the combination of carbamoyl phosphate and ornithine into citrulline. All arginine biosynthetic genes are regulated by the ArgR repressor protein, and co-repressed by free arginine. Thus any manipulation to enhance arginine biosynthesis requires derepression of the arginine regulon. In addition to deregulation, we investigate the role of overproduction of citrulline used recombinant integration and deletion of genes from the chromosome to produce an E. coli strain capable of stable rather than transient overproduction of citrulline. Further metabolic engineering focused on increasing the flow of nitrogen (as ammonia) into both the bacterial cells and into the arginine biosynthesis pathway. The combinatorial metabolic engineering of E. coli resulted in strains with at least six fold improvement in citrulline yields compared to wildtype.

**SYM-12-01**

**MAPPING HUMAN HISTORY: FROM OUR EARLIEST ANCESTORS TO COMPLEX ADMIXTURE**

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The ability to define the evolutionary history of modern humans, and in parallel the evolution of human adaptation and disease, has been dramatically advanced by the ability to contrast and compare patterns of DNA sequence variation between contemporary populations. Analyses of these patterns of variation suggest that the most divergent human lineages derive from the ancient people that once populated the most southern region of Africa. The southern African click-speaking Khoesan people not only present with the most divergent human genomes to date, but carry important genetic signatures about all modern human history. Our genomic data suggests that southern Africa is home to the oldest genetic human lineages, spanning around 170 thousand years. We show that the region was once home to a much larger diversity of modern humans with distinct foraging histories, we confirm the presence of these genomic signatures within the first successful DNA extraction from ancient skeletal human remains from the region, we provide the first genetic-based evidence for significant human evolution in the region around the Last Glacial Maximum, we demonstrate the presence of genetic signatures that define human adaptation from hunter-gatherer to agriculturalist, while demonstrating that largely extinct ancient human lineages may be carried in the DNA of contemporary modern day populations.
**(SYM-12-02)**

**THE SUCCESS OF WHOLE EXOME SEQUENCING DIAGNOSIS IN A LARGE COHORT OF PATIENTS WITH MENDELIAN DISORDERS**

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Molecular diagnosis in patients with Mendelian disorders has been improved significantly by advances in next generation sequencing (NGS). NGS has resulted in an enhanced mutation identification, genetic management and in some cases the creation of novel therapies. Mendelian disorders are most amenable to diagnosis though whole exome sequencing (WES) as the coding 2% of the genome is highly enriched for disease-causing mutations. WES was performed to identify the genetic cause of inherited diseases which are not identifiable from clinical genetics clinics in New South Wales and for whom molecular diagnosis was known. This cohort is phenotypically heterogeneous, however the majority of patients presented with intellectual disability (82%) consistent with population frequency and diagnostic importance. Other diagnoses included skeletal dysplasias (14%), retinitis pigmentosa (8%), haematological disorders (8%), seizures (3%), metabolic conditions (3%), and developmental syndromes (5%). DNA from anonymized exons and splice sites, untranslated regions, and the mitochondrial genome were captured with a Nextera extended exon kit on Illumina HiSeq 2500 sequencers. Genomic data was annotated with the Variant Effect Predictor (VEP) and common variants were excluded based on population polymorphism data and impact on protein function using the GEMINI (Genome MINING) software. The most likely inheritance model was applied to each family based on pedigree analysis and indication for referral and in consanguineous families, regions of homozygosity were identified and applied as an additional filter. Mutation pathogenicity scoring systems were applied including PROVEAN and CADD scores for missense and other mutations. We present an overview of the techniques and outcomes including a preliminary cost-effectiveness analysis. Initial results from 13 families show a diagnostic success rate of approximately 25% for mutations in known disease-related genes, and a likely novel disease gene for intellectual disability involved in neurite growth in a consanguineous family.

**(SYM-12-03)**

**TARGETED SEQUENCING REVEALS FINE DETAILS OF THE HUMAN GENOME**

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The human transcriptome is crowded with a diversity of coding and noncoding genes. We have developed a technique of RNA Capture Sequencing (CaptureSeq) that uses oligonucleotide probes to target and capture any gene of interest for focused sequencing. Targeted sequencing enables more sensitive gene discovery, more precise quantification of gene abundance, and more accurate isoform assembly. We applied these advantages of CaptureSeq to annotate long noncoding RNAs, profile the aberrant expression and splicing of cancer genes, discover new genes in empty disease-associated genome regions, analyse rare and transient RNA species and dissect novel splicing pathways. A deep analysis of transcription on chromosome 21 using CaptureSeq infers a immense size and diversity to the human transcriptome and, by comparison to the syntenic regions of the mouse genome, provides insight into the evolutionary forces that shape the mammalian transcriptional landscape.

**(SYM-12-04)**

**UBIQUITOUS LINE1 SOMATIC MOSAICISM IN HIPPOCAMPAL NEURONS REVEALED BY SINGLE CELL SEQUENCING**

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LINE-1 retrotransposons compose approximately 17% of the human genome sequence, with some elements retaining the capacity to replicate by a copy and paste mechanism known as retrotransposition. LINE-1 replication results in the insertion of up to 6kb of DNA containing functional DNA elements and can thus contribute to diseasew related elements. LINE-1 retrotransposition is a critical to neuronal function. LINE-1 retrotransposition events which preferentially target genomic loci critical to neuronal function.

**(SYM-12-05)**

**CHROMATINIZED PKC-THETA DIRECTLY REGULATES INDUCIBLE GENES IN EPITHELIAL TO MESENCHYAL TRANSITION AND BREAST CANCER STEM CELLS**

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Epithelial to mesenchymal transition (EMT) is a key step in cancer progression and the process of metastasis that creates a reservoir for cancer stem cells (CSCs), and is associated with highly aggressive traits. As well as driving metastasis, these CSCs, or precursor metastatic cells, play a pivotal role in therapeutic resistance and relapse in breast cancer patients. Global changes in the epigenetic landscape are a hallmark of cancer, but there is little knowledge of the chromatin landscape of CSCs. In particular, the contributions of the epigenetic mechanisms underpinning genes in CSC formation are at its infancy. Inducible and non-inducible in vitro human breast CSC models are amenable to detailed epigenetic analysis were used to study epigenetic marks on CSCs. Chromatin Immunoprecipitation (ChIP) and sequential chip assays were performed utilizing pharmacological blockade and siRNA strategies to identify the epigenetic tags that contribute to inducible CSC gene regulation. Intriguingly, we show for the first time that the recently discovered novel class of chromatin associated PKC enzymes distinctly control inducible gene expression programs in CSC subsets. Our ChIP sequencing identified unique genes bound to PKCs in the CSCs. Additionally, our finding exemplifies PKC driven CSC gene regulation. Collectively, this work has allowed the identification of distinct transcriptional programs existing in cancer stem cells along with establishing novel genetic signatures underlying EMT and CSC process. Undoubtedly this study will help in cancer diagnostics and designing new cancer therapeutics in the future.
SYM-13-01
DYNAMIC MONOMER-DIMER EQUILIBRIUM OF G-PROTEIN-COUPLED RECEPTORS AS DETECTED BY SINGLE-MOLECULE TRACKING

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Receptor dimerization is important for many signaling pathways. However, the monomer-dimer equilibrium has hardly been fully characterized for any receptors in the live-cell plasma membrane (PM) with a 2D-equilibrium constant as well as association/dissociation rate constants. In particular, the existence and function of dimers of class-A G-protein-coupled receptors (GPCRs) have been controversial. We determined the dynamic equilibrium for prototypical GPCRs, N-formyl peptide receptor (FPR) and beta2 adrenergic receptor (AR), by developing a “super-quantitation” method based on single fluorescnet-molecule imaging-tracking. For FPR and AR at 37°C, we obtained equilibrium constants of 3.6 and 1.6 copies/square-microns, dissociation rate constants of 11.0 and 12.6/s (90- and 80-ms dimer lifetimes), and 2D-association rate constants of 3.1 and 7.9 [copies/square-microns/s], respectively. Under physiological expression levels of 2.1 and 260 copies/square-microns [6,000 and 740,000 copies/cell] for FPR in human neutrophils and AR in dog heart cells, respectively, 42 and 95% of the molecules are expected to exist as dimers in the live-cell PM at any moment. Three research groups independently examined four class-A GPCRs (M1 muscarinic receptor, formyl-peptide receptor, and beta1- and beta2-adrenergic receptors) using single-molecule tracking, and found that they all exist as both monomers and homodimers, dynamically interconverting between each other (Hern et al. 2010; Kasai et al. 2011; Calebiro et al. 2013). These results suggest that transient homodimerization might be a general feature of the class-A GPCRs. Although the amino-acid sequences of GPCRs changed through evolution, they maintained their physical property of forming transient homodimers, in addition to their seven membrane-spanning structures, suggesting that dynamic homodimers might be critical for some of their important functions.

SYM-13-02
ONE E3 LIGASE TARGETS TWO KEY CONTROL POINTS IN CHOLESTEROL SYNTHESIS

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The mevalonate pathway is used by cells to produce sterol and non-sterol metabolites and is subject to tight metabolic regulation. We recently reported that squalene monoxygenase (SM), an enzyme controlling a rate-limiting step in cholesterol biosynthesis, is subject to cholesterol-dependent proteasomal degradation. However, the E3-ubiquitin ligase (E3) mediating this effect was not established. Using a candidate approach, we identify the E3 membrane-associated RING finger 6 (MARCH6, also known as TEB4) as the ligase controlling degradation of SM. We find that MARCH6 and SM physically interact, and consistent with MARCH6 acting as an E3, its over-expression reduces SM abundance in a RING-dependent manner. Reciprocally, knockdown of MARCH6 increases the level of SM protein and prevents its cholesterol-regulated degradation. Additionally, this increases cell-associated SM activity, but is unexpectedly accompanied by increased flux upstream of SM. Prompted by this observation, we found that knockdown of MARCH6 also controls the level of HMGR in hepatocytes and model cell-lines. In conclusion, MARCH6 controls abundance of both SM and HMGR, establishing it as a major regulator of flux through the cholesterol synthesis pathway.

SYM-13-03
PTRF/CAVIN-1 NEUTRALISES CAVEOLIN-1 IN PROSTATE CANCER MEDIATED BY EXTRACELLULAR VESICLE CONTENT

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Prostate cancer is one of the most common causes of cancer-death in men, mainly due to aggressive tumour metastasis. Caveolin-1 has a complex role in prostate cancer and has been suggested to be a potential biomarker and therapeutic target. As mature caveolin-1 resides in caveolae, invaginated lipid raft domains at the plasma membrane, caveolae have been suggested as a tumour-promoting signalling platform in prostate cancer. However, epidemiological studies reported the down-regulation of cavin-1, -2 and -3 in several cancers including prostate cancer. We recently showed that caveolin-1 in clinical prostate cancer specimens are not co-expressed with the adapter protein PTRF/cavin-1 also known as polymerase I and transcript release factor (PTRF; Moon et al. 2013 Oncogene). Furthermore, ectopic expression of PTRF/cavin-1 attenuated tumour growth and metastasis in orthotopic xenograft mice. To decipher the mechanism, we investigated the role of extracellular vesicles released by prostate cancer cells (also termed exosomes). Here we report the effects of PTRF/cavin-1 expression on extracellular vesicle content and function, and provide initial results on the mechanism in tumour microenvironments of metastatic sites.

SYM-13-04
A FEMALE-GAMETOCYTE-SPECIFIC ABC TRANSPORTER PLAYS A ROLE IN LIPID METABOLISM IN THE MALARIA PARASITE

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ATP-binding cassette (ABC) transporters serve a variety of physiological functions as well as playing key roles in drug resistance. The genome of the human malaria parasite, Plasmodium falciparum, encodes multiple members of this family, one of which, gABC22, is transcribed predominantly in the gametocyte stage of the parasite. Using gene deletion and tagging, we investigated the expression, localization and function of this protein. gABC22 is found in a single dot-like lipid-rich structure within female, but not male, gametocytes. gABC22 knock-out cell lines produce more gametocytes of both sexes. cGMP levels in the trophozoite stage increase upon deletion of the gABC22 gene. By contrast, cholesteryl esters, di- and triacylglycerols are significantly reduced in gABC22 knock-out gametocyte stages. We propose a role for gABC22 in the regulation of gametocyte numbers and in the accumulation of neutral lipids, which are likely important for parasite development in the insect stages of the parasite lifecycle.
SYM-13-05
CALPAIN: AN OVERDRIVE SWITCH FOR DYSFERLIN MEMBRANE REPAIR
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Plasma membrane repair following damage is critical for cell survival. Membrane repair is hypothesised to be mediated by calcium-dependent fusion of vesicles at the site of injury, which provide the membrane required for repair. The protein dysferlin is a mediator of calcium-dependent muscle membrane-repair, with mutations in dysferlin causing a progressive muscular dystrophy. Dysferlin is the putative calcium sensor for vesicle fusion-mediated membrane repair. Our research reveals that the calcium-dependent proteases, calpains, cleave dysferlin following membrane injury, releasing a 72kdDa C-terminal mini-dysferlin in a calcium-dependent manner. As only the C-terminus of dysferlin is identifiable at plasma membrane lesions, we hypothesised that mini-dysferlin is specifically recruited to injury sites. While calpain-1 is maximally active with <50μM Ca²⁺, calpain-2 requires super-physiological calcium concentrations (>200μM) for activation. Dysferlin cleavage requires ≥200μM extracellular calcium, indicating calpain-2 may preferentially regulate dysferlin behavior in membrane repair. Calpain-1 and -2 recruitment temporally precedes dysferlin at sites of injury, consistent with a role as primary mediators of dysferlin-dependent membrane repair. We have refined the calpain-cleavage of dysferlin to the alternately spliced exon 40a. An anti-exon 40a antibody reveals exon 40a-containing dysferlin recruits to sites of membrane injury, consistent with our hypothesis that mini-dysferlin is the dysferlin species at sites of injury. Other ferlin family members are enzymatically cleaved by calpains, also releasing C-terminal fragments. Calpain-cleaved mini-ferlins are structurally similar to the classical mediators of neurotransmitter vesicle fusion, syntaptogamins. We are currently investigating the role of calpain cleavage as a functional modulator of ferlins, which, with dysferlin we hypothesise confers a specialized role in vesicle fusion-mediated membrane repair.

SYM-14-01
RHO REGULATORS IN SYNAPESE DEVELOPMENT AND DISEASE
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Mutations in genes encoding Rho regulators have been found to underlie various forms of intellectual disability (ID). Oligophrenin-1 (OPHN1), which encodes a Rho-GAP, was the first identified Rho-linked ID gene. It was initially identified by the analysis of a balanced translocation t(X;12) observed in a female patient with mild ID. Subsequent studies revealed the presence of OPHN1 mutations in families with ID associated with cerebellar hypoplasia and ventriculomegaly. Most OPHN1 mutations identified to date have been shown, or predicted, to result in OPHN1 loss-of-function; however, the pathophysiological role of OPHN1 remains poorly understood. By temporally and spatially manipulating OPHN1 gene expression, we found that during early development postsynaptic OPHN1 plays a key role in activity-dependent maturation and plasticity of excitatory synapses, indicating the involvement of OPHN1 in normal activity-driven glutamatergic synapse development. More recently, we obtained evidence that OPHN1 plays also a critical role in mediating mGLUR-LTD in CA1 hippocampal neurons. mGLUR-LTD induction elicits rapid dendritic OPHN1 synthesis, which is dependent on mGLUR1 activation. This response is essential for mGLUR-LTD, as acute blockade of OPHN1 synthesis impedes LTD. mGLUR-induced OPHN1 mediates LTD and associated persistent decreases in surface AMPARs via interactions with Endophilin-A2/3. Importantly, this role of OPHN1 is separable from its effects on basal synaptic strength, which require OPHN1’s Rho-GAP activity and interaction with Homer1b/c. Thus, our data unveil a multifunctional role for OPHN1 at CA1 synapses. Independent of its role in activity driven glutamatergic synapse development, regulated OPHN1 synthesis plays a role in mGLUR-dependent LTD. As such, our findings provide insight into the cellular basis by which mutations in OPHN1 could contribute to the behavioral and cognitive deficits in OPHN1 patients.

SYM-14-02
UBIQUITINATION AND ENDOSOMAL TRAFFICKING OF THE TUMOR SUPPRESSOR PTEN DURING BRAIN DEVELOPMENT
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PTen controls a signalling axis that is implicated to regulate cell proliferation, growth, survival, migration and metabolism. In the brain loss of PTen function results in macroencephaly (enlarged brain) and behavioural abnormalities that have been linked to autism spectrum disorders. PTen is a phosphatase that negatively regulates the PI3K/Akt pathway, and through this enzymatic function it is anti-angiogenic, but the majority of mutations linked to autism retain PTen phosphatase activity. This suggests that PTen dysfunction in neurodevelopmental disorders can be caused by separate signalling networks outside of the PI3K/Akt pathway. Here we report the control of PTen activity and signalling specificity during the cell cycle by Ndfip1 and Rab5 GTPase regulation of PTen spatial distribution. Genetic deletion of Ndfip1 resulted in a loss of PTen nuclear compartmentalisation and increased cell proliferation. Cells lacking nuclear PTen have dysregulated levels of PI3K and cyclin D1 that were found to drive cell proliferation. In vivo, transgene expression of Ndfip1 in the developing brain increased nuclear PTen and lengthened the cell cycle of neuronal progenitors, resulting in microencephaly. Our results show that local partitioning of PTen from the cytoplasm to the nucleus represents a key mechanism contributing to the specificity of PTen signalling during cell division and brain development.

SYM-14-03
SYNAPTIC ACTIVITY CONTROLS THE FLUX OF AXONAL RETROGRADE CARRIERS IN PERIPHERAL AND CENTRAL NEURONS
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Although axonal retrograde transport of neurotrophins and their receptors carry survival information to the cell body, how presynaptic activity relates to changes in the survival signal is currently unknown. We used atoxic cholera toxin B-subunit (CTB), a known neuronal retrograde marker to investigate the relationship between synaptic activity and retrograde transport. We found that CTB enters motor nerve terminals in specific regions distinct from active zones at the amphibian and Drosophila neuromuscular junction. In cultured hippocampal neurons, CTB is internalised into early endosomes and small vesicles, morphologically indistinguishable from synaptic vesicles. Using microfluidic chambers we show that a proportion of the CTB-positive retrograde carriers were also TrkB-positive confirming that CTB labelled a population of signalling endosomes. Raising presynaptic activity through pharmacological and genetic modifications increases the flux of CTB-positive axonal retrograde carriers in hippocampal neurons grown in microfluidic devices and in Drosophila motor axons. Moreover, there is a significant increase in the number of small carriers (<100nm diameter), which cannot be resolved by conventional confocal microscopy. These small carriers were frequently observed to cluster together with larger, functionally distinct carriers. Together our data point to a clear link between presynaptic activity and the number and type of retrograde carriers, and suggest a connection between synaptic vesicle recycling and the generation of retrograde carriers carrying survival cues. These data further highlight the need for high-resolution techniques to fully understand the nature and relationship of between synaptic activity and retrograde trafficking in neurons.
NEURAL CELL ADHESION MOLECULE 2 GUIDES NEURONAL DEVELOPMENT BY INDUCING SUBMEMBRANE CALCIUM SPIKES

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Cell adhesion molecules are proteins at the cell surface of neurons, which help neurons to navigate in the complex brain environment during development. Changes in expression of the Neural Cell Adhesion Molecule 2 (NCAM2) have been proposed to contribute to neurodevelopmental disorders in humans. The role of NCAM2 in neuronal differentiation remains, however, poorly understood. Calcium serves as a critical secondary signaling messenger in neurons, which regulates a number of processes in growing neurons, including cytoskeleton remodeling and gene expression. Using genetically encoded Ca^{2+} reporters, we can analyze intracellular Ca^{2+} levels in different subcellular compartments of neurons in a real-time manner. We show that activation of NCAM2 at the neuronal cell surface induces submembrane [Ca^{2+}] spikes, which depend on the L-type voltage-dependent Ca^{2+} channels (VDCCs) and require activation of the protein tyrosine kinase c-Src. We also demonstrate that clustering of NCAM2 induces L-type VDCC- and c-Src-dependent activation of CaMKII. NCAM2-dependent submembrane [Ca^{2+}] spikes localize to the bases of filopodia. NCAM2 activation increases density of filopodia along neurites, neurite branching and outgrowth in L-type VDCC-, c-Src- and CaMKII-dependent manner. Our results therefore indicate that NCAM2 promotes filopodia formation and neurite branching by inducing submembrane Ca^{2+} influx and CaMKII activation. Changes in NCAM2 expression in Down syndrome and autistic patients may therefore contribute to abnormal neurite branching observed in these disorders.

ENDOSOMAL SORTING OF THE ALZHEIMER’S DISEASE B-SECRETASE, BACE1

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BACE1 (β-Secretase) plays a critical role in the initial cleavage of the amyloid precursor protein (APP) to produce amyloid β-peptides (Aβ) that are the major component of the amyloid plaques in Alzheimer’s disease (AD). Recent evidence has implicated endosomal dysfunction as an early neuropathologic feature in AD pathogenesis. The impairment of trafficking routes could contribute to an imbalance in endosomal recycling, signalling and turnover, hence the importance in understanding the regulation of the endo-lysosomal system in AD. We have previously shown that BACE1 is rapidly internalized by the AP2/clathrin dependent pathway and traffics to the early endosomes and Rab11-positive, juxtanuclear recycling endosomes in a number of cell lines; very little internalised BACE1 is transported to the trans-Golgi network. Here we assess the mechanisms that regulate endosomal sorting of BACE1. Depletion of Rab11 resulted in increased dynamic tubules containing BACE1 emanating from intracellular structures towards the cell surface and a block in internalized BACE1 transport from the recycling endosomes to the cell surface. Depletion of SNX4, a machinery component that mediates biogenesis of transport carriers from early endosomes to recycling endosomes resulted in both BACE1 and transferrin receptor redirected to the lysosomes. We have also explored a role for the cytoplasmic DISLL motif of BACE1 in early endosome-to-recycling endosome trafficking. Using antibody internalization assays, our results indicate that the phosphorylation status of serine498 influences the rate of trafficking of BACE1 to the recycling endosomes. Together these findings identify the signals and machinery that influence endosomal sorting of BACE1. ¹ Chia et al. (2013) Traffic, 14, 997-1013.
SYM-15-01

GLYCINE METABOLISM IN PHOTORESPiration

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CO2 fixation by leaf photosynthesis is accompanied by CO2 liberation caused by photorespiration and day respiration. While day respiratory CO2 release is quantitatively minor, photorespiratory CO2 production is near 25% of gross CO2 production under standard conditions and thus represents a major metabolic flux in the light. Despite this importance, very little is known about the regulation of the photorespiratory pathway. Here, I will present data obtained in sunflower leaves during experiments designed to pick short-term effects of CO2/O2 conditions on glycine metabolism, using quantitative NMR analyses and metabolomics.

SYM-15-02

THE HEAT IS ON: TEMPERATURE TRUMPS CO2 IN A BOREAL CONIFER

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Rising atmospheric CO2 concentrations are leading to warmer temperatures, with the greatest temperature increases expected at high latitudes. Determining how ecologically dominant species will respond to these rapid changes in climate is critical for predicting future atmosphere-vegetation feedbacks that affect the carbon cycle. We grew Norway spruce (Picea abies), an important boreal conifer, under a range of future climate scenarios (including ambient and elevated CO2 combined with ambient, +4°C and +8°C warming treatments) and measured growth, as well as temperature responses of photosynthesis, dark respiration and light respiration. Growth temperature effects were stronger than the effects of elevated CO2 and respiration acclimated to a greater degree than photosynthesis. Neglecting acclimation of light respiration to temperature significantly altered the conclusions drawn regarding how well photosynthesis acclimates to future climates, emphasizing the need to better understand how light respiration is affected by rising temperatures and CO2 concentrations.

SYM-15-03

ADVANCES IN MODIFYING LEAF CO2-FIXATION RATES

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The rate of photosynthetic carbon assimilation in plants is often limited by the catalytic rate of the CO2-fixing enzyme Rubisco. In nature there is significant natural diversity in catalytic performance of different Rubisco isoforms. Identifying the sequence elements in better performing isoforms (C, C, C-C) intermediate species, offering the unique opportunity to examine the evolution of C photosynthesis in the grasses. A robust phylogeny of the tribe, constructed with nuclear and plastid markers, showed two independent origins of C photosynthesis, and that C (photo)synthetic intermediacy evolved separately from the two C lineages. From this information, we chose the C species Neurachne annularis, the C species N. munroi, and the C-C intermediate N. minor to study the molecular evolution of C photosynthesis in the group. We have focused on the enzyme carbonic anhydrase (CA) as this enzyme catalyses the first step in the C pathway. In leaf tissues of the three Neurachne species, cDNAs encoding three distinct CA isoforms (CA1, CA2, CA3) have been isolated. Genomic DNA analysis has indicated that only two genes encode the three isoforms, with CA2 and CA3 products of alternative splicing. This is the only known example of alternative splicing for a plant CA. Reporter constructs using GFP showed that in all three species the CA1 and CA2 isoforms localise to the cytosol and chloroplast, respectively. Resolution of the intracellular location of CA3 is currently underway. In leaves of N. minor and N. munroi, transcript abundance of CA1 is at least 10 times higher than for CA2 or CA3. Transcript levels in N. annularis are currently being determined. This information will give insights into what it took to evolve C grass, and what may need to be considered in current attempts to introduce a C pathway into C crop plants.

SYM-15-04

THE NEURACHNINAE: A NEW PARADIGM TO INVESTIGATE THE EVOLUTION OF C4 PHOTOSYNTHESIS

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The Neurachninae is the only grass lineage known to contain closely related C, C, and C-C intermediate species, offering the unique opportunity to examine the evolution of C photosynthesis in the grasses. A robust phylogeny of the tribe, constructed with nuclear and plastid markers, showed two independent origins of C photosynthesis, and that C (photo)synthetic intermediacy evolved separately from the two C lineages. From this information, we chose the C species Neurachne annularis, the C species N. munroi, and the C-C intermediate N. minor to study the molecular evolution of C photosynthesis in the group. We have focused on the enzyme carbonic anhydrase (CA) as this enzyme catalyses the first step in the C pathway. In leaf tissues of the three Neurachne species, cDNAs encoding three distinct CA isoforms (CA1, CA2, CA3) have been isolated. Genomic DNA analysis has indicated that only two genes encode the three isoforms, with CA2 and CA3 products of alternative splicing. This is the only known example of alternative splicing for a plant CA. Reporter constructs using GFP showed that in all three species the CA1 and CA2 isoforms localise to the cytosol and chloroplast, respectively. Resolution of the intracellular location of CA3 is currently underway. In leaves of N. minor and N. munroi, transcript abundance of CA1 is at least 10 times higher than for CA2 or CA3. Transcript levels in N. annularis are currently being determined. This information will give insights into what it took to evolve a C grass, and what may need to be considered in current attempts to introduce a C pathway into C crop plants.
Notably, this pattern did not change over the growing season and reached minima at the end of the growing season.

Changes in the photosynthetic responses of Malus domestica cv. Red Gala leaves to internal CO₂ concentrations (A/Ci) at a range of leaf temperatures from 15 to 40°C were followed at regular intervals across the growing season in field-grown trees. Light and CO₂-saturated photosynthesis (Amax), maximum rates of Ribulose 1,5-Bisphosphate (RuBP) carboxylation (Vcmax) and maximum rates of electron transport (Jmax) were all highest in spring, although Amax was maximal at 30°C and Vcmax and Jmax maximal at 40°C. All attributes declined in late summer and reached minima at the end of the growing season.

Marked changes in the temperature-dependency of Vcmax and Jmax occurred across the season, related to changes in ambient conditions, particularly high temperatures late in the season. The reduction in photosynthesis at these high ambient temperatures was partly attributable, up to 60%, to a stomatal limitation. However, the transition chloroplast CO₂ concentration from carboxylation-limited assimilation to RuBP regeneration-limited assimilation increased with increasing leaf temperatures. This analysis revealed that for the Apple leaves, the non-stomatal limitations were from limited RuBP regeneration below 25°C and by limited RuBP carboxylation at temperatures above. Notably, this pattern did not change over the growing season and appeared to be an intrinsic property of the photosynthetic process. There were some seasonal changes in the temperature-dependency of Vcmax and Jmax, notably by a change in the activation energy, especially in the late season when the high ambient temperatures occurred. This suggested, at least for the Apple leaves, that the temperature-dependency of the carboxylation and regeneration processes was not uniform over the growing season and needs to be considered in models of photosynthesis.

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The allocation of net primary production into different plant components is an important process affecting the lifetime of carbon in ecosystems, resource use and productivity by plants. In global models that aim to predict the effect of climate change on the land carbon sink, the representation of biomass allocation has been identified as a major source of uncertainty. Existing models use simplified allometric models to determine allocation, but it is not known to what extent these models apply equally well to different plant functional types, vegetation types, or different climatic conditions. One major roadblock to improving our understanding of biomass allocation is the availability of a comprehensive dataset. Here we present an individual-level data set capturing ontogenetic patterns of biomass allocation in woody plant species from biomes across the globe. The data were compiled from published and unpublished sources, focussing on studies where: i) measurements were for individual plants rather than whole stands; ii) individuals spanning a range of sizes were measured; and iii) size was estimated directly, i.e. not indirectly via allometric equations. We included both wild and artificially grown plants. The dataset contains many plant size metrics and biomass measurements, including total leaf area, plant height and stem cross-section, crown size, dry mass of leaf, stem, roots, and several others. Overall, the dataset contains 202907 measurements collected in 112 different studies, from 20642 individuals across 165 species. We demonstrate how BAAD can be used to study effects of plant functional type on allometric scaling relationships in woody plants.

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SYM-16-04

EXPERIMENTAL MANIPULATION OF TEMPERATURE, SALINITY AND OSMOTIC STRESS TO DETERMINE GERMINATION THRESHOLDS OF COASTAL PLANT SEEDS

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Seed-mediated colonisation into new environments depends on the ability of seeds to germinate and survive under new environmental conditions. Coastal plants may achieve long-distance dispersal through oceanic transport of seeds; however, this is likely to result in exposure to increased levels of environmental stress. Here, manipulations of temperature, salinity and osmotic stress were used to investigate the post-dispersal germination capacity of seeds from multiple populations of two coastal plants. Seeds were most sensitive to salinity (exhibited reduced germination in, or recovery from, saline conditions) at non-optimal temperatures (5 and 20 °C for Spyridium globulosum; 10 and 25 °C for Ficinia nodosa; the extremes at which germination occurred). However, salt tolerance of seeds of both species varied between populations, suggesting that some populations are likely to have greater recruitment capacity than others and this may impact the adaptive capacity and persistence of species under environmental change. Furthermore, investigation of the relative reduction in germination caused by water stress and ion toxicity revealed that the impact of these stressors is also moderated by temperature. Collectively, these results indicate that the interaction between temperature, ion toxicity, osmotic stress and within-species variation can affect germination in new environments. In particular, salt sensitivity may be intensified as species disperse to, or experience, environments of non-optimal temperature.

SYM-16-05

GENETIC VARIATION IN WATER USE EFFICIENCY (WUE) AND RESPONSE TO DROUGHT IN SELECTED WHEAT LANDRACES

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Drought is the main abiotic constraint to agricultural productivity in Australia. Climate change-induced droughts and temperature increases are expected to reduce wheat production by over 29% globally. This scenario is compounded by stagnating yields, increasing irrigation costs and scarcity of water for irrigation. The most plausible way to curtail this is developing water responsive and water use efficient (WUE) wheat cultivars. Mesophyll conductance (gm) and leaf intrinsic water use efficiency WUEi have been reported as appropriate indices for selecting water use efficient genotypes. The main goal of this study was to determine genetic variation in WUEi of wheat landraces as well as assessing responses of gm, stomatal conductance (gs), leaf water potential, photosynthetic rate (A) and WUEi to drought stress. A, gs and gm were measured under non-limiting conditions for 41 wheat landraces from the Watkins collection and 9 commercial genotypes, indicating significant genotypic differences, with gm values ranging between 0.6-1.4 mmol m-2s-1 and WUEi ranged from 40-70 μmolmol-1. Three landraces and two commercial genotypes were subjected to a slowly-developing drought and monitored intensively. Drought increased WUEi and reduced A, gs, and leaf water potential, while there was no effect on gm. The genotypes varied in the time they took to start responding to the drought stress. The wide genetic variation in wheat landraces is a good resource for improving water efficiency in wheat.

SYM-17-01

EPIGENETIC MEMORY

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When somatic cell nuclei are transplanted to enucleated eggs, in many cases there is a complete reversal of cell differentiation. However, in some cases the reversal is incomplete and the cells of the resulting nuclear transplantation show memory of their previous specialized gene expression. This memory reflects the epigenetic status of the donor cell nucleus. Current work is aimed at elucidating the basis of this epigenetic memory and of the epigenetic changes undergone by transplanted nuclei. It has recently been discovered, as cells pass through mitosis, their chromatin changes so as to provide a brief opportunity for accelerated reprogramming of genes.

SYM-17-02

RECONFIGURATION OF THE DNA METHYLOME DURING CELLULAR REPROGRAMMING

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Induced pluripotent stem (iPS) cells offer immense potential for regenerative medicine and studies of disease and development. Somatic cell reprogramming involves epigenomic reconfiguration, conferring iPS cells with characteristics similar to embryonic stem (ES) cells. Large-scale restructuring of the epigenome takes place throughout mammalian cellular differentiation and development, and whole genome bisulfite sequencing has revealed that the DNA methyloomes of pluripotent and differentiated human cells are very distinct. Through generation of whole genome single base resolution maps of DNA methylation in multiple human iPS and ES cell lines, progenitor somatic cells, and differentiated iPS and ES cells, we identified significant reprogramming variability in iPS cells, including somatic memory and aberrant reprogramming of DNA methylation. Moreover, these epigenomic abnormalities in iPS cells persisted through differentiation at a high frequency, providing an iPS cell reprogramming signature that is maintained after differentiation. Recent studies indicate that the method of reprogramming can affect the frequency of epigenomic abnormalities, prompting us to investigate the influence of reprogramming to a primed or naive state upon the epigenomic aberrations in iPS cells, and to characterize the temporal dynamics of methylome reconfiguration during reprogramming.
SYM-17-03

DIRECTED DIFFERENTIATION OF HUMAN PLURIPOTENT CELLS TO FORM A SELF-ORGANISING KIDNEY

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During normal embryogenesis, the mammalian kidney is formed from two cellular compartments that interact with each other via a series of cell-Cell and ligand-receptor driven interactions. Signals from the nephron progenitors drive dichotomous branching of the ureteric epithelium to form the collecting duct system of the organ. Conversely, signals from the epithelium support a nephron progenitor population to self-renew (and hence continued support of epithelial branching) as well as commitment to nephron formation. Drawing from our understanding of the embryonic processes involved in specifying posterior primitive streak (phase 1), intermediate mesoderm (phase 2) and metanephric kidney development (phase 3) in the mouse, we have now successfully directed the differentiation of human pluripotent stem cells (hESC and iPSC) to simultaneously form both of these key nephron progenitor lineages in vitro (Takasato et al, 2014). This was achieved using a fully chemically-defined monolayer culture with stepwise induction of each phase of development. By day 14 of differentiation, we observed synchronous induction of elongating epithelial PAX2+/GATA3+/Ecad+ UE together with a surrounding mesenchymal PAX2+/Six3+/WT1+ MM. Given the evidence for the presence of both progenitor populations in the dish, cultures were dissociated and then reaggregated to form a pellet and cultured as an organoid culture. Within these cultured aggregates, self-organizing events were observed, generating renal vesicles, proximal tubules and collecting ducts. The capacity for such populations to undergo self-organization in vitro provides the potential for disease modeling using patient-derived iPSC cell lines, the generation of cells for tissue/organ bioengineering and in the longer term the possibility of cellular therapies.

SYM-17-04

GENERATING CELL LINEAGES DURING HUMAN CARDIOVASCULAR DEVELOPMENT

Skelton R.J.P.1, Anderson D.A.1, Karavendzas K.1, Ararasatnam D.1, Qian L.1, Elefanty A.G.1, Stanley E.G.1, Mummery C.L.1 and Elliott D.A.2
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NKX2-5 marks early multipotent progenitor cells and fully differentiated cardiomyocytes in the developing cardiovascular system. We have examined the potential of NKX2-5 expressing cells to contribute to various cardiovascular lineages by targeting GFP to the NKX2-5 locus in human pluripotent stem cells. In early stages of mesodermal differentiation, NKX2-5low CD34pos cells can be identified that further differentiate primarily to an NKX2-5neg CD34pos population. At the same early stage of differentiation, NKX2-5low CD34neg cells can also be identified and further differentiated to generate an NKX2-5neg CD34pos population. The CD34pos cells derived from both NKX2-5low populations are proliferative, predominantly KDRpos, and express the endothelial markers TEK, CDHS and PECAM1. The NKX2-5low CD34neg cells can also give rise to an NKX2-5high population that is contractile in culture and expresses the cardiac markers MYH6, TNNT2 and MYL2. This indicates the potency of early NKX2-5pos cells to give rise to both endothelial and cardiac lineages in vitro. At later stages of differentiation NKX2-5low cells lose their ability to transition to a CD34pos or NKX2-5high fate and progressively become NKX2-5neg. Later stage NKX2-5high cells retain contractility and cardiac gene expression, with few cells transitioning away from this phenotype. This indicates a loss of potency in NKX2-5pos cells during in vitro differentiation. We are currently investigating the potential of later stage NKX2-5low cells to differentiate to smooth muscle and fibroblast lineages. These studies demonstrate restricted potency of NKX2-5 expressing cells during the course of in vitro mesodermal differentiation.

SYM-17-05

THE MIXL1 TRANSCRIPTIONAL NETWORK IN CELL PRIMING AND MORPHOGENETIC CELL MOVEMENTS

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Successful application of stem cells to replacement cell therapies and regenerative medicine rely on understanding the molecular signals governing the balance between pluripotency and differentiation. Traditional views of lineage commitment advocate progressive restrictions in cell potency along one of the three primary tissue lineages driven by key lineage-specific transcription factors. However, these views have been challenged by recent studies suggesting multiple multi-potent progenitor stages [1], a pluripotency spectrum and priming for differentiation [reviewed by 2]. This research examines the role of one such early differentiation marker, Mixl1, implicated in regulation of mesoderm and endoderm differentiation and/or morphogenesis [3,4]. Using a global transcriptional analysis, by microarray of mouse ES cells expressing cells during the course of in vitro mesodermal differentiation.

SYM-17-08

THE IDENTIFICATION AND VALIDATION OF PROTEIN:LIGAND COMPLEXES: RECONCILING THEORY AND EXPERIMENT

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Understanding how a given ligand, such as a pharmaceutical drug molecule, interacts with its receptor whether this is a therapeutic target, metabolizing enzyme or carrier molecule is central to the success of drug design and understanding the pharmacokinetic properties of molecules more generally. In particular, knowing the 3-dimensional structure of a ligand:receptor complex in detail is central to computational drug discovery. The three dimensional structure of a molecule cannot be observed directly, however. Instead, data from X-ray diffraction and/or Nuclear Magnetic Resonance (NMR) is combined with atomic interaction parameters in order to generate a structural model. In the case of high resolution X-ray diffraction data the experimental density term dominates. However, at medium to low resolution (> 1.5 Å) the quality of the final structure is primarily determined by the reliability of the molecular interaction and geometric parameters used during refinement. Highly optimized and well-validated interaction parameters (force fields) are available for common biomolecules such as proteins, but not for most drug-like ligands or enzyme co-factors. This frequently leads to errors in the proposed structures, the misinterpretation of experimental data. The presentation will focus on what extent existing structures can be trusted and how molecular dynamics simulations in combination with free energy calculations can be used to validate and correct the structures of ligand:protein complexes. In addition, how molecular simulation techniques more generally can be used to gain insight into the process associated with ligand-receptor interactions not assessable to experiment will be discussed.
SYM-18-02

LIGAND DISCOVERY BY TARGET SIMILARITY SEARCH

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Most widely used, successful methods for computer-assisted inhibitor design are either based on known ligands (ligand-based) or structures of known receptors (structure-based). The ligand-based approach requires information of existing ligands for a specific target (receptor protein). The requirement makes it difficult to discover new classes of ligands. A structure-based approach can discover novel inhibitors by docking to a receptor structure and ranking them according to a scoring function. The performance of existing scoring functions, however, remains hit or miss at best. Here, we present a new technique that discovers novel inhibitors from ligands that bind to proteins structurally similar to the target receptor. The method yields a success rate of 30% in top 1% prediction compared to 13% by AUTODOCK vina, 10% by DOCK 6 and 3% by random prediction in the 103-target DUD benchmark where each active compounds has an average of 36 inactive decoys.

SYM-18-03

DNA CLAMPS: STRUCTURE, DYNAMICS AND DRUG DISCOVERY

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DNA clamps are torus-shaped proteins that serve as processivity-promoting factors in DNA replication in humans, bacteria and some viruses. By encircling DNA, they act as a mobile tether for other proteins in DNA replication and repair. Bacterial DNA clamps are a critical component of the DNA polymerase III holoenzyme and several other enzymes in DNA replication and repair. The conservation of bacterial DNA clamp interactions with its partner proteins and its moderate cellular abundance (300–600 per cell) suggest it as an attractive antibiotic drug target. Because the DNA clamp coordinates multiple essential cellular events through binding interactions with many different protein partners at the same binding site, we hypothesize that drugs targeting bacterial DNA clamps will present a much greater challenge to the evolution of resistance. I will describe the structure and dynamics of DNA clamps generally, and our efforts to inhibit the bacterial DNA clamps through fragment screening and medicinal chemistry.

SYM-18-04

INHIBITOR PEPTIDE DESIGN – IMPROVING AFFINITY WITHOUT LOSING SPECIFICITY

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A challenge in the development of inhibitors of intracellular targets is to achieve specificity and high affinity for those targets. Here peptides, that can mimic specific protein-protein interaction surfaces, can provide the answer, so long as cell permeability and stability issues are addressed. Here we describe progress in developing a specific inhibitor of the Grb7-SH2 domain involved in cancer progression. Grb7 is an adapter protein, aberrantly co-overexpressed with erbB-2 and identified as an independent prognostic marker in breast cancer. Grb7 signals the activation of erbB-2 which plays a key role in deregulated cell growth in cancer. Grb7 also mediates signalling pathways from focal adhesion kinase (FAK) exacerbating cell migration and the metastatic potential of cells. It is thus a prime target for the development of novel anti-cancer therapies. We have characterised a non-phosphorylated cyclic peptide (G7-18NATE) that is a specific inhibitor of Grb7 and inhibits cellular growth and migration in cancer cell lines. X-ray crystallographic structure determination of the G7-18NATE/Grb7-SH2 domain complex and binding studies using SPR revealed the basis of affinity and specificity of the peptide. Here we describe how this information has been used to successfully design second generation bicyclic peptides that show enhanced binding without loss of specificity. These peptides can be coupled to cell penetrating peptide sequences to be taken up by cells, lowering cell growth and migration. We anticipate that these Grb7 inhibitor peptides will form the basis of novel therapeutics that can be used in conjunction with existing therapies against breast cancer.

SYM-18-05

TARGETING CELL DEATH PATHWAYS TO TREAT PARASITIC INFECTIONS

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Schistosomiasis is one of the world’s most wide-spread and serious infectious diseases which is caused by parasitic flatworms. Here we describe the identification and characterization of a Bcl-2-regulated apoptosis pathway in schistosome worms, and provide evidence that this could provide a novel target drugs to treat schistosomiasis. Our genomic, biochemical and cell-based mechanistic studies provide evidence for an apoptotic pathway similar to that in humans, including BH3-only proteins that are inhibited by pro-survival Bcl-2-like molecules, and Bax/Bak-like proteins that facilitate mitochondrial outer-membrane permeabilization. A class of drugs called “BH3-mimetics” that directly antagonise Bcl-2 proteins are currently being successfully trialled in the treatment of cancer. We have biochemical data showing that a schistosome pro-survival protein, sBcl-2, also binds to BH3-mimetic drugs, and a crystal structure showing that it binds in an almost identical manner to the way it binds its human counterparts. Moreover, we have also shown that BH3-mimetic drugs have a striking effect on schistosome worm physiology, in particular, on their capacity to produce viable eggs, as well as their overall morphology and behaviour. As these results suggest BH3-mimetics could eventually provide a new treatment option for schistosomiasis, we screened libraries of BH3-mimetic-like compounds and identified a family of new lead compounds for future drug development efforts. We will also describe current efforts to identify and characterise Bcl-2 family proteins in other infectious organisms, including parasitic nematodes which cause many diseases in humans and livestock.
Understanding mechanisms of cell motility is of considerable importance if we are to develop a therapeutic approach to the metastatic spread of cancer. Cell motility driven by the lamellipodium is dependent on rapid cycles of formation of branched actin filaments. The branches result from the use of Arp2/3 to initiate new filament formation from the sides of other actin filaments. In metastasizing cells actin filaments either consist of actin only or they exist as a co-polymer with tropomyosin. The Arp2/3 branched filament network consists of actin alone. We now report the discovery of a second type of filament containing both actin and tropomyosin which is involved in lamellipodium function. These filaments are defined specifically by the tropomyosin isoforms Tm5a/b which are almost entirely located in this cellular compartment. Knock down of Tm5a/b has no impact on the overt structure of the lamellipodium, but results in a substantial decrease in cell motility. To extend these findings we have investigated the role of tropomyosin in lamellipodium-driven cell motility. One filament type drives rapid rounds of membrane protrusion while the other is required to convert a protrusion into net forward movement of the cell. In addition, this new finding further supports the growing body of literature that the specificity of function of an actin filament is decided in part by the associated tropomyosin supports the growing body of literature that the specificity of function of an actin filament is decided in part by the associated tropomyosin.
Cells use actomyosin contractility to move through three-dimensional (3D) extracellular matrix. Contractility affects the type of protrusions cells use to migrate in 3D, but the mechanisms are unclear. We report that contractility generates high-pressure lobopodial protrusions in primary human fibroblasts migrating in a 3D matrix. In lobopodial cells, the nucleus and associated intracellular membranes physically divide the cytoplasm into high and low pressure forward and rear compartments, respectively. The compartmentalized elevated pressure in lobopodial cells corresponded with the ability of the nucleus to accelerate periodically away from the trailing edge. Vimentin intermediate filaments formed a contractility-dependent complex with actomyosin and the nuclear envelope-cytoskeleton linker protein nesprin 3. Knocking-down nesprin 3 abolished the independent movement of the nucleus and slowed 3D migration of the cells in a manner similar to treatment with blebbistatin. Additionally, nesprin 3 knock-down reduced and equalized pressure between the front and back cytoplasmic compartments of cells to lamellipodia-based migration. Inhibition of myosin II in front of the nucleus by the local application of blebbistatin immediately reduced hydraulic pressure, while applying the drug behind the nucleus had no effect. Hence, the nucleus can act as a piston that is pulled forward by actomyosin contractility, acting on nesprin 3 to increase the hydrostatic pressure between the nucleus and the leading edge to drive lamellipodia-independent 3D cell migration. This mechanism is required for the efficient migration of primary human cells through physiological 3D matrix and highlights the importance of physical factors such as matrix microenvironment and pressure in mammalian cellular functions.

**SYM-20-02**

**BOOSTING THE POWER OF ENDOGENOUS PHOSPHATASES TO REDUCE INFLAMMATION IN ASTHMA**

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Asthma is a clinically and socioeconomically significant disease driven by inflammation. Corticosteroids are the mainstay of anti-inflammatory therapy in respiratory disease. Although corticosteroids have proven clinical efficacy in asthma, many asthmatic inflammatory conditions (e.g. infection, exacerbation) are not responsive to them. Corticosteroid insensitivity can range from relative corticosteroid insensitivity to steroid resistance, as seen in severe asthma. Thus, alternative anti-inflammatory strategies are urgently needed and enhancing the function of endogenous phosphatases, especially protein phosphatase 2A (PP2A), offer great promise. PP2A is a master controller of multiple inflammatory signalling pathways. It is an exciting target for anti-cancer therapy and has more recently emerged as a druggable target in respiratory disease. Our recent work aims to uncover the molecular mechanisms of PP2A regulation and pharmacological modulation in cellular models of asthmatic inflammation and relative corticosteroid insensitivity in vitro in A549 lung epithelial cells. We show that the classical PP2A activator - FTY720 - increases PP2A activity and significantly represses IL-6 and IL-8 mRNA expression and cytokine secretion. Similar PP2A-mediated repression of inflammation is also achieved with AAL(S), a chiral analog of FTY720 devoid of sphingosine 1-phosphate agonism. In combination with corticosteroids, FTY720 achieves significant repression of IL-6 and IL-8 secretion with lower concentrations of steroids. Excitingly, PP2A activators can also alleviate corticosteroid resistance because FTY720 can still repress cytokine secretion even when corticosteroid insensitivity occurs after exposure to bacterial ligands (TLR2 agonist; Pam3CSK4). Thus, PP2A activators offer a non-steroidal anti-inflammatory alternative and/or corticosteroid-sparing adjunct approach in asthmatic inflammation. These studies highlight the potential of boosting the power of endogenous phosphatases as novel anti-inflammatory strategies to combat asthmatic inflammation, particularly in the context of corticosteroid insensitivity.

**SYM-20-03**

**SIGNAL TRANSDUCTION BY PP2A: REGULATING THE REGULATOR**

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Protein phosphatase 2A (PP2A) is a family of enzymes that represent a major portion of Ser/Thr phosphatase activity in most cells, and play a key role in signalling. Significantly, deregulation of PP2A has been associated with a variety of disorders, including cancer and Alzheimer disease. As such, there is growing interest in targeting PP2A or PP2A regulators for therapeutic purpose. However, the regulation of PP2A is highly complex, and to date, remains poorly understood. Of particular interest, methylation of PP2A plays an important role in modulating PP2A biogenesis and substrate specificity. Here, we show that genetic-dietary interactions that alter folate metabolism can induce changes in PP2A methylation, resulting in PP2A dysfunction in vivo. We also present novel cellular findings showing that PP2A methylation can also be modulated by activation of specific signal transduction cascades. Our findings establish a link between metabolic and signalling pathways that can Underlie the deregulation of PP2A and could contribute to pathogenic mechanisms in many diseases.
SYM-20-04

OXYGEN-DEPENDENT HYDROXYLATION BY FACTOR INHIBITING HIF (FIH) REGULATES THE TRPV3 ION CHANNEL


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Factor Inhibiting HIF (FIH) is an oxygen-dependent asparaginyl hydroxylase that regulates the transcriptional activity of hypoxia-inducible factors (HIFs). Several proteins containing ankyrin repeat domains have been characterised as substrates of FIH, although there is little evidence for a functional consequence of hydroxylation on these substrates. FIH knockout mice display metabolic phenotypes distinct from predicted HIF-dependent changes, supporting a role for non-HIF substrates. Bioinformatic analysis identified a number of the transient receptor potential (TRP) ligand gated ion channels, which contain ankyrin repeat domains, as likely substrates of FIH. We demonstrate that the transient receptor potential vanilloid 3 (TRPV3) channel is hydroxylated by FIH on asparagine 242 within the cytoplasmic ankyrin repeat domain. Hypoxia, FIH inhibitors and mutation of the target asparagine all potentiate TRPV3-mediated current, without altering TRPV3 protein levels, indicating that oxygen-dependent hydroxylation inhibits TRPV3 activity. This novel mechanism of channel regulation by oxygen-dependent asparaginyl hydroxylation is likely to extend to other ion channels.

SYM-20-05

SRC KINASE DETERMINES THE DYNAMIC EXCHANGE OF THE DOCKING PROTEIN NEDD9 AT FOCAL ADHESIONS

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Dynamic exchange of molecules between the cytoplasm and integrin-based focal adhesions provides a rapid response system for modulating cell adhesion. Increased residencies time of molecules that regulate adhesion turnover contributes to adhesion stability, ultimately determining migration speed across 2-dimensional surfaces. In the present study, we test the role of Src kinase in regulating dynamic exchange of the focal adhesion protein NEDD9/HEF1/Cas-L. Using either chemical inhibition or fibroblasts genetically null for Src together with Fluorescence Recovery After Photobleaching (FRAP), we find that Src significantly reduces NEDD9 exchange at focal adhesions. Analysis of NEDD9 mutant constructs with the two major Src interacting domains disabled revealed the greatest effects were due to the NEDD9 SH2 binding domain. This correlated with a significant change in 2D migratory speed. Given the emerging role of NEDD9 as a regulator of focal adhesion stability, the time of NEDD9 association at the focal adhesions is key in modulating rates of migration and invasion. Our study suggests that Src kinase activity determines NEDD9 exchange at focal adhesions and may similarly modulate other focal adhesion-targeted Src substrates to regulate cell migration.

SYM-21-01

Professor Ian Chubb AC, Australia’s Chief Scientist

Professor Ian Chubb commenced in the role of Chief Scientist on 23 May 2011. Prior to that, Professor Chubb was Vice-Chancellor of the Australian National University from January 2001 to March 2011; Vice-Chancellor of Flinders University of South Australia for six years and the Senior Deputy Vice-Chancellor of Monash University for two years while simultaneously the Foundation Dean of the Faculty of Business and Economics for 16 months.

He was Chair of the Higher Education Council (the Commonwealth Government’s peak advisory body on higher education) from September 1990 to December 1994 and was, until mid-1994, the Deputy Chair of the National Board of Employment, Education and Training (the Commonwealth’s peak advisory body on all matters related to the Employment, Education and Training portfolio).

From January 1986 to September 1990, Professor Chubb was the Deputy Vice-Chancellor of the University of Wollongong and an Honorary Professor of Biology. During the period 1978-1985 he was an academic member of the School of Medicine of Flinders University. Before that he was at Oxford University where, during the period 1971-1977, he was a Wellcome Foundation Scholar, a Junior Research Fellow of St John’s College, and a Royal Society Research Fellow. He spent 1969-1971 as a JF & C Heymans Research Fellow at the University of Ghent, Belgium.

Professor Chubb’s research focused on the neurosciences and was supported by the National Health and Medical Research Council, the Australian Research Grants Scheme and by various Foundations. Professor Chubb was President of the Australian Vice-Chancellors’ Committee (AVCC) for 2000 and 2001, Vice-President for 1998 and 1999 and an elected member, or member, of the Board of the AVCC between 1996 and 2006. From January 2000 to December 2001 Professor Chubb was a member of the Prime Minister’s Science, Engineering and Innovation Council (PMSEIC). He serves, or has served, on numerous other awards and Committees related to his university or Commonwealth responsibilities – in universities and in the public and private sectors.

In 1999 Professor Chubb was made an Officer of the Order of Australia (AO) for “service to the development of higher education policy and its implementation at state, national and international levels, as an administrator in the tertiary education sector, and to research particularly in the field of neuroscience”. In 2006 he was made a Companion (AC) in the order for “service to higher education, including research and development policy in the pursuit of advancing the national interest socially, economically, culturally and environmentally, and to the facilitation of a knowledge-based global economy”. In 2000, Professor Chubb was awarded a Doctor of Science (h.c.) from Flinders University. He was the ACT’s Australian of the Year in 2011. Also in 2011 he was awarded a Doctor of Literature (h.c.) from Charles Darwin University and a Doctor of the University (h.c.) from the Australian National University; and in 2012 he was awarded a Doctor of Laws (h.c.) from Monash University.

SYM-21-02

LEARNING FROM PRODUCTIVE FAILURE

Kapur M.

National Institute of Education, Singapore.

In my talk, I will advance a theoretically- and empirically-grounded case for designing for and learning from failure, and instantiate it in a learning design I call Productive Failure (PF). I will describe the key mechanisms and the design principles of PF, and report findings from an on-going program of research in the real ecologies of Singapore Math classrooms. I will end by deriving implications for learning and the design of instruction.
FREE ENERGY RADIO: INTERVIEWS WITH SCIENTISTS DEVELOP A PICTURE OF SCIENTISTS' PERSONAL AND CAREER MOTIVATIONS

Rowland S.
University of Queensland.

It is well established that students in high schools and universities view science and scientists as "other", and that students have few mechanisms that they can use to access information about "who" a real scientist is, and "what" they do all day. This paucity of information affects the willingness of students to enter a career in the sciences. In this project we aim to address this information gap by producing a podcasted series of student-driven interviews with working scientists that we call "Free Energy". The interviews were conducted in a working radio studio. The discussion was semi-structured, with a deliberate focus on career trajectory and motivation for career choices. We present a short description of the Free Energy project and an analysis of the interviews, including (i) an examination of the character traits the scientists exhibited and (ii) a discussion of the factors that influenced the scientists’ career choices. Perhaps most importantly, we also examine how the student interviewers recontextualised their goals and expectations as a result of their involvement in Free Energy.

METABOLIC PATHWAYHS REQUIRED FOR POLLEN EXINE FORMATION IN PLANTS

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The lipidic structure, exine (outer pollen wall) surrounding pollen grains, plays a key protective role for the male gametophyte and pollen grain development. We recently identified and characterized tapetum-expressed enzymes/genes such as the cytochrome P450 members: CYP704B2, CYP703A3; fatty acid reductases Defective Pollen Wall (DPW) in rice and Male Sterile 2 (MS2) in Arabidopsis essential for pollen exine formation. CYP704s and CYP703s catalyze fatty acid hydroxylation and are conserved in land plants. The DPW and MS2 proteins localize predominantly in plastids, and reduced fatty acids to alcohols, representing a novel function of plastids in fatty acids reduction. In addition, we identified two ABC Transporters, Post-meiotic Deficient Anther1/OsABCG15 from rice, and WBC27/AtABCG26 from Arabidopsis which are assumed to transport sporopollenin precursors from tapetal cells to microspore surface for pollen wall formation. Moreover, the bHLH transcription factors ABORTED MICROSPORES in Arabidopsis and its ortholog Tapetum Degeneration Retardation in rice function as master regulators coordinating the expression of genes relative to callose dissociation, fatty acids elongation, formation of phenolic compounds, and lipidic transport putatively involved in sporopollenin precursor synthesis.

HYBRID MIMICS AND THE MECHANISM OF HYBRID VIGOR

Wang L.1, Groszmann M.1, Greaves I.K.1, Wu L.1, Peacock W.J.1,2 and Eichten S.R.1
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The basis of hybrid vigor has eluded understanding for over a century despite its wide use in agriculture. In Arabidopsis we have produced, in only a few generations, Hybrid Mimic lines which breed true for the major traits of the F1 hybrid, increased biomass and increased seed yield. In the hybrid these characteristics are limited to the first generation. The hybrid and the Hybrid Mimics have larger leaves than their parents resulting from an increased number of photosynthetic cells. In some hybrids and in one Hybrid Mimic line cell size is increased, also contributing to the extra photosynthetic capacity of the hybrid. Many genes differentially expressed in the F1 hybrid relative to the parents are also differentially expressed in the Hybrid Mimic lines. Epigenetic processes such as Trans Chromosomal Methylation can affect transcription levels of these genes emphasising the importance of interactions between the genome and epigenome in hybrid vigor.

IDENTIFICATION AND CLASSIFICATION OF DNA METHYLATION VARIATION ACROSS MAIZE

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DNA methylation is a chromatin mark that can play a role in silencing transposons and in some cases, genes. While DNA methylation is often described as an epigenetic modification, it is more properly described as a chromatin mark that may be caused by genetic variation or can be independent of genetic variation resulting in true epialleles that may impact heritable variation regardless of genetic state. This distinction is important as genetically-controlled methylation variants will be accounted for in GWAS approaches while epialleles are likely to be missed. Genome-wide profiling of DNA methylation in diverse maize (Zea mays) lines has identified several thousand differentially methylated regions (DMRs) across genotypes. In some cases DNA methylation changes are strongly associated with local genetic variation such as polymorphic transposable element insertions resulting in DMRs in flanking genomic regions. From this, the phenotypic importance of DMRs may also depend on the amount of genetic diversity between samples. To explore this, current work using Brachypodium distachyon as a model grass allow for large-scale bisulfite sequencing of accessions that are genetically similar, yet are established in unique environments. This natural system will allow for epiallele identification and association to adaptive phenotypes between populations. The ability to separate genetically-controlled DNA methylation and true epialleles, combined with unique natural systems to study adaptive phenotypes, will allow the contribution of epigenetics to plant function to be elucidated.
SYM-22-04

ARABIDOPSIS AND AGRICULTURE - WHAT CAN WE LEARN FROM A WEED?

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The face of modern agriculture changed when the Arabidopsis genome sequence was published in 2000. This was the first plant genome to be sequenced and detailed the genes necessary to specify a plant with all its biological processes (about 25,000 genes, the same number as humans). This genome sequence enables research to start with identification of a gene in Arabidopsis which can then be used to find similar genes in a crop species. There are many examples of Arabidopsis being used to unravel the mechanism of plant processes, for example identification of the genes important for making oils in canola or for nutrient uptake such as phosphorus. The increased performance of hybrids relative to their parents is one of the remaining puzzles of plant biology. Hybrids were important for Australia’s most famous wheat breeder Farrer, after whom this lecture is named. Farrer was one of the first wheat breeders to use hybrids and his famous “Federation” wheat was a hybrid between “Yandilla” (itself a hybrid) and “Purple Straw.” By 1910, “Federation” was the most widely grown Australian wheat and remained so for another 15 years, increasing the acreage of wheat in NSW by four fold. I will describe our approaches to understanding the mechanisms of hybrid vigour using Arabidopsis as a model. We have used Arabidopsis ecotypes which have near identical genomes to determine that epigenetic mechanisms including a novel process, Trans Chromosomal Methylation, are involved in the generation of hybrid vigour. Using recurrent selection we have produced Hybrid Mimics that approach F1 hybrids in size and seed yield. Genes that are similarly regulated in the F1 hybrids and Hybrid Mimics identify pathways that may be critical for hybrid vigour. The work I am presenting results from a team effort of Li Wang, Michael Groszmann, Ian Greaves, Marina Trigueros, Rebecca Gonzales-Bayon, Limin Wu, Aihua Wang, Bjorg Sherman and Jim Peacock.

SYM-23-02

THE HISTONE ACETYLTRANSFERASE MOZ IN CARIOVASCULAR AND CRANIOFACIAL DEVELOPMENT

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All cells of the mammalian body develop from a single cell, the fertilised oocyte. The diverse cell types are genetically identical and their morphological and functional differences are thought to be due to differences in the pattern and timing of gene expression. While gene expression is regulated by DNA-binding transcription factors, these typically act in more than one cell lineage and thus additional mechanisms are required to confer lineage specificity. A major role is played by chromatin-mediated mechanisms, which, at each lineage decision point, render gene loci either available or unavailable for transcriptional activation, thus limiting transcriptional activation to the set of genes appropriate for a specific cell lineage. Acetylation of histone lysine residues is a chromatin modification that is typically associated with open chromatin and the active transcriptional state. The MYST family of lysine acetyltransferases comprises about 30% of the mammalian genome’s capacity to regulate chromatin structure and gene expression at the level of histone acetylation. Lack of individual MYST family members leads either to genome-wide changes in chromatin structure and gene expression, or to changes in transcription at a restricted number of gene loci. Accordingly, mutation of MYST family members causes either early embryonic lethality or specific defects in embryonic patterning and organ development. Monocytic leukaemia zinc finger protein (MOZ, MYST3, KAT6A) is the target of recurrent translocations causing an aggressive form of leukaemia. Congruently, it is required for the development of haematopoietic stem cells. Importantly, MOZ has a number of critical roles during embryonic patterning and organ development. Mouse embryos lacking MOZ have an extensive anterior homeotic transformation affecting 19 body segments as a result of failure of appropriate transcription and acetylation at Hox gene loci. This pivotal role in transcriptional activation extends to a small number of other genes including genes that regulate palate, aortic arch and cardiac development, such as T-box genes. Using expression profiling and genetic interaction studies, we have identified new target genes of MOZ, indicating that MOZ is an important regulator of embryonic patterning genes.

SYM-23-03

THE ADPKD GENE PKD1 REGULATES LYMPHATIC VASCULAR MORPHOGENESIS DURING DEVELOPMENT

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The initial process that gives rise to lymphatic vessels during development is the sprouting of precursor cells from embryonic veins, which involves a number of well-studied growth factor and transcription factor pathways. Less well understood are the genes and pathways that regulate the ongoing elaboration of individual vessels to form a network. This is driven by cellular processes including polarisation, coordinated migration, adhesion, mixing, regression and cell shape rearrangements. Using a genetic screen in zebrafish, we identified the mutant lymphatic and cardiac defects 1 (lyc1) which has reduced lymphatic vessel development. We demonstrate that a mutation in polycystic kidney disease 1a (PKD1) encodes POLYCYSTIN1, and is the most frequently mutated gene in autosomal dominant polycystic kidney disease (ADPKD). We find that the initial sprouting of lymphatic precursors is normal in lyc1 mutants, but that ongoing lymphatic endothelial precursor cell migration fails. Loss of Pkd1 in germline and endothelial knockout mice also has no effect on initial sprouting of precursor cells from veins but leads to a failure of the morphogenesis of the subcutaneous lymphatic vascular network. This is associated with defective cell polarity and altered adherens junctions in individual sprouting vessels. Recent findings suggest that Pkd1 likely acts through modulation of the PCD pathway in vascular development, as it does in kidney morphogenesis. This unexpected role for Pkd1 serves as a new entry point to understand vessel morphogenesis in development and disease.
DISCOVERY OF A NEUROPROTECTIVE CHEMICAL

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A phenotypic screen performed in search of neuroprotective chemicals queried the activities of 1,000 synthetic chemicals. Compounds were pooled into groups of ten and administered into the brain tissue of living mice. One week later animals were sacrificed and scored for protective activity of hippocampal neurons. The seventh pool scored for protective activity of hippocampal neurons. The seventh pool was found to be neuroprotective.

A chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. The chemical, designated pool seven, was found to be neuroprotective. 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SYM-24-03
CHASING THE STRUCTURAL DIVERSITY OF AUTOTRANSPORTER PROTEINS
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Our current understanding of bacterial physiology stems primarily from studies on free swimming (planktonic) bacteria, which have also been the basis for the development of all current antibiotics. However, more than 99% of bacteria exist in aggregated communities and biofilms, which are complex structures associated with their enhanced resistance to antimicrobials, chemical detergents and immune factors. Many persistent and chronic bacterial infections are associated with the formation of aggregates and biofilms, which are difficult to treat, including respiratory and urinary tract infections (UTIs), infections on medical devices and infections of the ear, gums and heart. Thus, an increased understanding of the mechanisms employed by bacteria to form biofilms is essential for the development of strategies to combat these persistent and intrinsically resistant communities. One mechanism of bacterial aggregation and biofilm formation involves the expression of self-associating surface-located autotransporter (AT) proteins. Our work focuses on investigating the structural diversity of AT proteins to understand their mechanism of action. We have recently elucidated the structuraltand crystal structures of Antigen 43 (Ag43), an AT protein from uropathogenic E. coli (UPEC) that self associates forming bacterial aggregates and biofilms. Our studies have shown how Ag43's L-shaped structure drives the formation of cell aggregates via a molecular Velcro-like mechanism. Furthermore, our recent studies on other AT proteins from E. coli pathotypes show unexpected structural diversity among this family of proteins, which results in different virulence functions. For example UpaB shares low sequence and structural similarity with Ag43, does not self associate to form bacterial aggregates, but binds extracellular matrix proteins (e.g. fibronectin) and increases bladder colonisation.

SYM-24-05
DESIGNED CYCLIC PEPTIDE TARGETING SPSB PROTEINS AS A POTENTIAL NEW CLASS OF ANT-INFECTION AGENT
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Inducible nitric-oxide synthase (iNOS) plays a critical role in the clearance of intracellular pathogens such as Leishmania major and Mycobacterium tuberculosis through nitric oxide (NO) production. iNOS is of the target of SPRY-domain containing SOCS box proteins (SPSBs), which direct this enzyme to ubiquitination and proteosomal degradation. iNOS contains a highly conserved pentapeptide motif (DINNN) in its N-terminal region that interacts strongly with the SPRY-domain containing SOCS box proteins (SPSBs). By inhibiting the iNOS-SPSB interaction, the intracellular lifetime of iNOS can be prolonged, which in turn enhances the clearance of chronic L. major parasites.1 We have implemented 19F NMR as a means of probing the environment of Trp residues in SPSB2 and gaining insight into the DINNN-SPSB2 interaction.4 Trp was chosen as the W207A mutation abrogates DINNN-SPSB2 interaction,4 and crystal structures show that W207 is in close proximity to the iNOS binding site. We replaced six Trp residues in SPSB2 with 5-F-Trp by utilizing a Trp auxotroph E. coli strain.1,2 NMR results clearly shows that the 5-F-Trp resonance of W207 is perturbed significantly upon binding with DINNN. This recently established 19F NMR strategy on SPSB2 has enabled us to rapidly identify potential inhibitors that block the DINNN-SPSB2 interaction. Here, we describe a disulfide-linked Ac-[cDINNNc]-NH2 peptide that binds to SPSB2 with high affinity, Kd ~4 nM, determined by surface plasmon resonance (SPR). Both ITC and NMR data indicate that this cyclic peptide binds to the same iNOS binding site and forms a strong hydrogen bond network with SPSB2. Evidence from cell-based assays showed that the SPSB2-DINNN interaction site was blocked by this cyclic peptide. This peptide will serve as a valuable starting point from which to generate a new class of anti-infective agents.

SYM-25-01
ENDOSOME BIOGENESIS AND THE REGULATION OF SIGNALLING
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The small GTPase Rab5 is a master regulator of endosome biogenesis and function. We have recently conducted a genome-wide RNAi screen for transferrin and EGF endocytosis, revealing design principles of the endosomal network. This is organized as a “funnel” whereby internalized cargo progresses from many, small early endosomes at the cell periphery to few, large endosomes at the cell centre over time. Ultimately, these large endosomes undergo conversion from Rab5 to Rab7. The genomic screen has further revealed that cells accurately regulate the number of EGF endosomes, their size, concentration of EGF and intracellular position. To investigate the functional consequences of such regulation, we performed a quantitative analysis of phosphorylated epidermal growth factor receptor (p-EGFR) internalized in endosomes. We found that p-EGFR is not randomly distributed but packaged at a constant mean level in endosomes. By mathematical modelling we found that such a mechanism serves to regulate the extent, duration and robustness of the signalling response.
SYM-25-02
THE ROLE OF RABS, RAB GAPs AND RAB GEFS IN INSULIN ACTION IN ADIPOCYTES
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Insulin regulation of glucose uptake into adipocytes plays an essential role in the regulation of whole body glucose metabolism and insulin sensitivity. This process is mediated via the canonical PI3K/Akt pathway, which regulates factors that control the translocation of intracellular vesicles containing GLUT4 glucose transporters (GSVs) leading to their fusion with the plasma membrane (PM). This translocation process is regulated by Rab GTPases and their regulatory proteins, RabGAPs and RabGEFs. The RabGAP AS160 is phosphorylated by Akt in response to insulin in fat cells and this is thought to inhibit its GAP activity toward the candidate Rabbs, Rab8, Rab10, and Rab14. AS160 binds to GSVs via its interaction with IRAP, a cargo protein found in GSVs. DENND4c, a Rab10 GEF, is also localized to GSVs in non-stimulated cells and we have preliminary evidence that AS160 and DENND4c are located on the same vesicles. In our recent studies we have found that insulin stimulates the phosphorylation of the Rab10 GEFs, DENND4a/c. DENND4a is an mTOR substrate that binds to mTOR. We are currently exploring the role of insulin regulated DENND4a phosphorylation in mTOR-regulated processes in the fat cell. Finally, we have generated Rab10 -/- mice and find that this is embryonic lethal and a subset of Rab10 +/- mice show exencephaly. We are currently analysing insulin action in Rab10 -/- MEFs that were differentiated into adipocytes. These studies provide new insights into how insulin regulates membrane trafficking processes in the adipocyte. Excitingly, the presence of a GAP and a GEF for the same Rab substrate together on the same vesicles gives rise to a novel hypothesis concerning regulated intracellular vesicle transport.

SYM-25-03
THE P53-INDUCED FACTOR EI24 INHIBITS NUCLEAR IMPORT THROUGH AN IMPORTIN B-BINDING-LIKE DOMAIN
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The etoposide-induced protein EI24 was initially identified as a p53-responsive, pro-apoptotic factor, but no clear function has been described. We used a non-biased proteomics approach to identify members of the Importin (IMP) family of nuclear transporters as interactors of EI24 and characterize an IMPb-binding-like (IBBL) domain within EI24. We show that EI24 can bind specifically to IMPb1 and IMPo2, but not other IMPs, and use a mutated IMPb1 derivative to show that EI24 binds to the same site on IMPb as the Impo IBBl. Ectopic expression of EI24 reduced the extent of IMPb1- or IMPo1-dependent nuclear protein import specifically, whereas specific alanine substitutions within the IBBL abrogated this activity, as determined by quantitative confocal laser scanning microscopic analysis. Induction of endogenous EI24 expression through etoposide treatment similarly inhibited nuclear import in a mouse embryonic fibroblast model. Thus, EI24 can bind specifically to IMPb1 and IMPo2 to impede their normal role in nuclear import, shedding new light on the cellular functions of EI24 and its tumour suppressor role.

SYM-25-04
FERLIN-IN OR FERLIN-OUT? CHARACTERISING THE SUBCELLULAR LOCALISATION OF THE FERLIN PROTEIN FAMILY
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The ferlin proteins are an ancient, conserved family of calcium-activated vesicle fusion proteins, present in the simplest eukaryotes. In humans, dysferlin mutations cause muscular dystrophy, otoferlin mutations cause a profound form of deafness, and myoferin has been implicated in cancer development; the remaining three mammalian ferlins (FerL1-4-6) are yet to be associated with disease. The defining structural features possessed by each of the ferlins are the six or seven calcium-dependent, lipid-binding C2 domains, believed to play important roles in calcium-activated vesicle fusion. Indeed both invertebrate and vertebrate ferlin animal models are united by pathologies linked to defects in calcium-activated vesicle fusion, suggesting ferlins share functional parallels with the classical mediators of secretory exocytosis, the synaptotagmins, bearing two C2 domains. We have previously shown that dysferlin localizes to the plasma membrane and to endosomal vesicles using luminal and cytoplasmic epitopes to verify membrane topology, but very little is currently understood regarding the subcellular localisation of the other ferlin family members. We hypothesize that different ferlins may regulate calcium-activated vesicle fusion within different compartments of the secretory pathway. Our first important goal, although seemingly elementary, is to unambiguously define the intrinsic cellular targeting of each ferlin, which represents a significant knowledge-gap in the ferlin field, and is essential information to begin to understand their function. Using flow cytometry and confocal microscopy, we show for the first time that some ferlins are plasma membrane ferlins, and some partition to intracellular membranes; just like synaptotagmins. We will now use our expertise with the latest technologies in super-resolution, 3D-structured illumination microscopy, to demonstrate the precise location to which each of the ferlins localizes within the cell.

SYM-25-05
SHEER STRESS TRIGGERS TRANSLATION OF TRPV4 CHANNELS TO THE CELL SURFACE
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Vascular endothelial cells are under constant shear stress from the flowing blood, and shear induces a release of factors that relax surrounding vascular smooth muscle cells, resulting in vasodilatation. Calcium signalling is believed to be a key element in endothelial mechanotransduction and vascular dilation. Ion permeable channels mediate many of these effects, however, the effect of shear stress on ion channel functions is poorly understood. Transient receptor potential (TRP) vanilloid 4 (TRPV4) channel, a polymodal calcium permeable channel, is expressed in vascular endothelial cells and activated by a range of stimuli including shear stress. TRPV4 has been shown to participate in controlling vascular tone including flow mediated dilatation. Here, we have characterised the nature of [Ca2+]i elevation in endothelial cells. We found that shear stress causes an proportional increase in [Ca2+]i that is mainly through TRPV4. Stimulation with shear stress showed a progressive recruitment of functional TRPV4 to the cell membrane, which is found to be through exocytosis, as inhibitors of “clathrin”, “dynamin” and “vesicle shuttling through the endoplasmic reticulum and golgi apparatus” prevented this effect. Additionally, this effect required an intact actin network and was blocked by inhibitors of the PI3K signalling pathway. TRPV4’s role in the control of vascular tone and recapitulation of our data in cultured HEK293, bovine aortic endothelial and human umbilical vein endothelial cells, suggests that this may be a general mechanism by which endothelial cells respond to changes in shear stress to regulate vascular dilation in response to blood flow.
SYM-26-01

effect of mhc-i tcr signal strength on lineage uncertainty and the time required for cd8 t cell positive selection in the thymus

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Immature CD4+8a (DP) thymocytes can differentiate into either mature CD4 T cells or mature CD8 T cells, with their ultimate developmental fate related to the MHC specificity of their TCR. However, thymocyte lineage fate is not signalled by TCR alone, but is determined by a dynamic interplay between TCR and cytokines during positive selection. As described by the kinetic signalling model, prolonged TCR engagements during positive selection induce Thpok expression and CD4 T cell differentiation, whereas disrupted TCR engagements allow intrathymic cytokines to induce Runx3 expression and CD8 T cell differentiation. As a result, positive selection of CD8 T cells consists of two distinct steps: (1) MHC class I-restricted thymocytes receive TCR signals but remain lineage-uncommitted; then (2) MHC class I TCR engagements cease and thymocytes are signalled by intrathymic cytokines to express Runx3 and differentiate into CD8 T cells. We have now examined the impact of TCR signal strength on the amount of time required for each component step of CD8 positive selection. We found that, compared to weak MHC class I TCR signals, strong MHC class I TCR signals had profound but opposite effects on each of the component steps of positive selection. Strong MHC class I TCR signals significantly prolonged lineage uncertainty in step 1 by delaying when thymocytes could be signalled by intrathymic cytokines to undergo CD8 lineage commitment. But, in step 2, strong MHC class I TCR signals then increased the responsiveness of thymocytes to intrathymic cytokines which accelerated the rapidity with which CD8 committed thymocytes became mature CD8 T cells. Thus, the small overall effect of TCR signal strength on CD8 T cell differentiation obscured profound, but opposite, effects of TCR signal strength on the component steps of MHC class i-specific positive selection in the thymus.

SYM-26-02

two-site interaction underpins trim25 activation of the rig-i anti-viral response

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The retinoic acid-inducible gene-1 (RIG-I)-like receptors are an important family of cytosolic viral RNA sensors. RIG-I recognizes short 5’-triphosphate base-paired viral RNA and is a critical mediator of the innate immune response against viruses such as influenza A, HIV and hepatitis C. This response requires a carefully orchestrated interaction with tripartite motif 25 (TRIM25). The binding of viral RNA and the hydrolysis of ATP induce a conformational change in RIG-I, which releases its tandem CARD domains for interaction with the TRIM25 B30.2 domain. TRIM25 then functions as an E3 ubiquitin ligase to stabilize the formation of a RIG-I tetramer and facilitate RIG-I interaction with MAVS (mitochondrial anti-viral signalling) at the mitochondrial membrane. The net result is expression of the type I and III interferon (IFNs) and the establishment of an anti-viral state. We have previously solved the crystal structure of the mouse TRIM25 B30.2 domain and identified key residues that are critical for the interaction with the RIG-I CARDs (site 1) (1). We have now identified a second CARD-binding site on the TRIM25 B30.2 domain that is revealed by removal of an N-terminal alpha-helix (inhibiting TRIM25 dimerization) (site 2). We provide biochemical evidence to suggest that both CARDs participate in this interaction and that a conformational change is required to expose a structurally-similar helix in CARD2. This suggests a model whereby the RIG-I CARDs interact with opposing sides of the TRIM25 B30.2 domain to form a higher order TRIM25/RIG-I complex that facilitates RIG-I tetramerisation. The characterization of a dual binding mode for the TRIM25 B30.2 domain is a first for the SPRY/B30.2 family and has broader implications. For instance, disease-causing mutations in the MEVF gene encoding Pyrin (TRIM20) map to its B30.2 domain in a region analogous to “site 2”. (1) D’ Cruz et al., biochem J, 2013.

SYM-26-03

defects in cytokine signaling pathways manifesting as human immune deficient diseases

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Intracellular signaling pathways induced by interactions between surface receptors and their cognate ligands facilitate the generation of successful immune responses. This underlies the efficient neutralization and clearance of pathogens, and the establishment of long-lived immunological memory. The corollary of this is that perturbations to signaling pathways compromise the differentiation and effector function of immune cells, underlying the clinical features of, and disease pathogenesis in, primary immunodeficiencies. We have been studying lymphocyte development, differentiation and effector function in individuals with loss-of-function mutations in Stat3, which causes autosomal dominant hyper-IgE syndrome and is characterized by susceptibility to infection with specific pathogens, impaired long-term humoral immunity and increased incidence of B-cell lymphoma. This has provided an opportunity to ascribe lineage-specific functions to Stat3, and Stat3-activating cytokines, and to identify defects in lymphocyte biology caused by Stat3-deficiency. Most importantly, these studies have provided mechanistic explanations for the clinical features of AD-HIES, thereby identifying molecules and signaling pathways that could be targeted for intervention to improve immune function in affected individuals.

SYM-26-04

the hidden guanylate cyclase function of irak3: a new trick from an immune protein


Interleukin-1 receptor associated kinase 3 (IRAK3) is a critical signaling mediator which acts as a negative regulator of inflammation by inhibiting downstream signaling in the innate immune response. The exact mechanism of action and the selectivity of IRAK3 is however still largely unclear. Using a bioinformatics guanylate cyclase (GC) homology search tool, novel candidate GCs were identified, including IRAK3. The new class of GCs contains a unique architecture, in which the GC domain is encapsulated within the kinase domain, thereby exhibiting a dual kinase-GC functionality. Here we show that recombinant IRAK3 protein is capable of producing cGMP at levels comparable to those produced by an archetypical dual kinase-GC, phytosulfokine receptor 1 (PSKR1). Alignment of homology models of the kinase domains of IRAK3 and PSKR1 reveal structural similarities between these two proteins. The uniformity in the structural topology of PSKR1 and IRAK3 may explain the observed GC activity in IRAK3. In separate experiments, HEK293T cells transfected with IRAK3 produced significantly higher levels of cGMP when compared to transfected control cells. Further in vitro studies are underway to investigate the functionality of IRAK3 in terms of its catalytic function in the presence of other signal components. The cGMP generated by the IRAK3 protein may be involved in its function and selectivity by modulating the binding and/or activity of nearby interacting proteins involved in the signaling pathway. These findings are providing insight into the hidden functions of IRAK3 and may assist in explaining the selectivity and functionality of IRAK3 in the inflammatory signaling cascade.
SYM-26-05

SOLUBLE HEPARAN SULFATE FRAGMENTS GENERATED BY HEPARANASE TRIGGER THE RELEASE OF PROINFLAMMATORY CYTOKINES THROUGH TLR-4

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Heparanase is a β-D-endoglucuronidase that cleaves heparan sulfate (HS), facilitating degradation of the extracellular matrix (ECM) and the release of HS-bound biomolecules including cytokines. The remodeling of the ECM by heparanase is important for various physiological and pathological processes, including inflammation, wound healing, tumour angiogenesis and metastasis. Although heparanase has been proposed to facilitate leukocyte migration through degradation of the ECM and basement membrane, its role in inflammation by regulating the expression and release of cytokines has not been fully defined. In this study, the role of heparanase in regulating the expression and release of cytokines from human and murine immune cells was examined. Human peripheral blood mononuclear cells treated ex vivo with heparanase resulted in the release of a range of pro-inflammatory cytokines including IL-1β, IL-6, IL-8, IL-10 and TNF. In addition, mouse splenocytes treated ex-vivo with heparanase resulted in the release of IL-6, MCP-1 and TNF. A similar pattern of cytokine release was also observed when cells were treated with soluble HS, indicating that heparanase-induced cytokine release could be mediated via the release of cell surface HS fragments. Due to soluble HS being able to signal through the Toll-like receptor (TLR) pathway, heparanase may promote the upregulation of cytokines through the generation of heparanase-cleaved fragments of HS. In support of this hypothesis, mouse spleen cells lacking the key TLR adaptor molecule MyD88 demonstrated an abolition of cytokine release after heparanase-stimulation. Furthermore, TLR4-deficient cells showed a decrease of cytokine release in response to heparanase treatment, suggesting that TLR4 is involved in this response. Consistent with these observations, the pathway involved in cytokine upregulation was identified as being MyD88-dependent. These data identify a new mechanism for heparanase in promoting the release of pro-inflammatory cytokines that is likely to be important in regulating cell migration and inflammation.

SYM-27-01

PANNEXIN 1 CHANNELS REGULATE CELL DISASSEMBLY DURING APOPTOSIS

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In humans, more than 20 billion cells will undergo programmed cell death (known as apoptosis) daily as part of normal turnover. It is crucial that dying cells are readily removed as their accumulation has been linked to numerous disease states including inflammation, autoimmunity and cancer. To aid efficient removal of dying cells, cells undergoing apoptosis often disassemble into smaller fragments (known as apoptotic bodies) that function as bite-size pieces for neighbouring cells to engulf. Although the generation of apoptotic bodies is a critical step during cell death, the mechanism underpinning this process is not well understood. To elucidate regulators of this process, we performed a flow cytometry-based drug screen and identified the ability of an antibiotic trovafloxacin to markedly enhance apoptotic bodies formation. Surprisingly, trovafloxacin also blocked the uptake of monomeric cyanine dye TO-PRO-3 by apoptotic cells, a process mediated by caspase-activated pannexin 1 (PANX1) channels. Through several lines of evidence, PANX1 was identified as an unexpected mammalian target of trovafloxacin and interference with PANX1 function during apoptosis enhanced apoptotic bodies formation. Mechanistically, inhibition of PANX1 activity during apoptosis promoted the formation of a novel string-like membrane protrusion (coined as apoptopodia) that facilitates the disassembly of apoptotic cells. Collectively, these findings identify PANX1 as a negative regulator of apoptotic cell disassembly, with important implications for the complex intercellular communication between apoptotic cell corpses and phagocytes.

SYM-27-02

A NOVEL MOUSE MODEL TO DISSECT THE MECHANISMS OF BCL-2 REGULATED CELL DEATH IN VIVO

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Over the last decade there has been considerable debate about how Bcl-2 pro-survival proteins regulate apoptosis. Several different models have been proposed, suggesting that the pro-survival proteins inhibit apoptosis through sequestration of “activator” Bcl-3-only proteins (Mode 1 / Direct activation model), and through interactions with Bax/Bak that prevent them from oligomerising on the outer mitochondrial membrane (Mode 2 / Indirect activation model). The evidence for both scenarios, based on in vitro studies, is very strong, however there are very few studies that have examined precisely how pro-survival proteins inhibit apoptosis in vivo. Here we describe a novel mouse model that provides a complete physiological readout of the interaction between Bcl-xL and Bak. These mice carry a germline point mutation in the BH3 domain of Bcl-xL, which biochemical and X-ray crystallographic studies (using both mouse and human proteins) demonstrate impaired binding to Bcl-xL, but not other pro-survival Bcl-2 family members. Mice carrying this mutation exhibit severe thrombocytopenia and significantly reduced thymocyte counts compared to wild-type animals. Other cell types such as fibroblasts from the mutant mice are also more sensitive to apoptotic stimuli and chemotherapeutics compared to those from wild-type animals. Hence, this model provides a powerful tool to study the mechanisms of cell death regulation in platelets and T-cells that are so poorly understood. Moreover, these mice extend previous genetic studies on apoptosis regulation as they provide precise mechanistic information on how the phenotypes are generated. Such information cannot readily be discerned from more conventional genetic approaches such as Bcl-2 pro-survival or pro-apoptotic protein knock-outs. The data we have to date demonstrates that inhibition of Bak by Bcl-xL (i.e Mode 2 / Indirect activation) is critical for survival of certain cell types in vivo.

SYM-27-03

NOVEL SIGNALS REGULATING AUTOPHAGY AND CELL DEATH

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During animal development excess or unnecessary cells are removed by programmed cell death (PCD). Most developmental PCD occurs by caspase-dependent apoptosis with other modes including autophagy and necrosis having context specific roles. To understand the mechanism and regulation of PCD during development we have been using Drosophila as a model. During metamorphosis obsolete larval tissues including the midgut and salivary glands are removed by PCD. Our studies have shown that midgut removal is not dependent on the apoptotic machinery, but requires autophagy. To understand the regulation and mechanism of autophagic PCD we have been investigating the roles of various growth signalling pathways and the requirement of the components of the starvation-induced autophagy pathway. Our data show that the signals that regulate starvation-induced autophagy, including TOR and PI3K, are also required to regulate autophagy during midgut removal. By systematic knockdown of Autophagy-related (Atg) genes in the midgut we found a specific requirement for distinct Atg genes during midgut PCD compared to those that are required during starvation-induced autophagy. These results and the roles of other signalling pathways involved in regulating autophagy during midgut PCD will be discussed. Given the dual roles of autophagy in cancer cell survival and cell death, our findings provide important insights into understanding the regulation and function of autophagy in cell death.
SYM-27-04

THE PROTONOPHORE CCCP INTERFERES WITH LYPOSOMAL DEGRADATION OF AUTOPHAGIC CARGO IN YEAST AND MAMMALIAN CELLS

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Mitophagy is a selective pathway, which targets and delivers mitochondria to the lysosomes for degradation. Depolarization of mitochondria by the protonophore CCCP is a strategy increasingly used to experimentally trigger not only mitophagy, but also bulk autophagy. Using live-cell fluorescence microscopy we find that treatment of HeLa cells with CCCP caused redistribution of mitochondrially-targeted dyes, including DiOC6, TMRM, MTR and MTG, from mitochondria to the cytosol, and subsequently to lysosomal compartments. Localization of mitochondrial dyes to lysosomal compartments was caused by retargeting of the dye, rather than delivery of mitochondrial components to the lysosome. We show that CCCP interfered with lysosomal function and autophagosomal degradation in both yeast and mammalian cells, inhibited starvation-induced mitophagy in mammalian cells, and blocked the induction of mitophagy in yeast cells. PARK2/Parkin-expressing mammalian cells treated with CCCP have been reported to undergo high levels of mitophagy and clearance of all mitochondria during extensive treatment with CCCP. Using correlative light and electron microscopy in PARK2-expressing HeLa cells, we show that mitochondrial remnants remained present in the cell after 24 h of CCCP treatment, although they were no longer easily identifiable as such due to morphological alterations. Our results show that CCCP inhibits autophagy at both the initiation and lysosomal degradation stages. In addition, our data demonstrate that caution should be taken when using organelle-specific dyes in conjunction with strategies affecting membrane potential.

SYM-27-05

EXAMINING THE ROLE OF MLKL IN NECROPTOTIC CELL DEATH

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Necrotic cell death has long been regarded as an uncontrolled consequence of chemical or mechanical insult. Recent work has revealed it to be intricately programmed and deliberate in certain scenarios like infection. This programmed necrosis or ‘necroptosis’ is triggered by a cascade of intracellular signals culminating in the phosphorylation of the pseudokinase Mixed Lineage Kinase domain-Like (MLKL). How MLKL goes on to cause cell death remains a strong topic of contention, with proposed mechanisms ranging from ion channel modulation to direct physical phospholipid bilayer disruption. Here, we show that the MLKL pseudokinase domain acts as a latch to restrain an N-terminal four-helix bundle (4HB) domain and that unleashing this domain results in formation of a high molecular weight, membrane localized protein complex and cell death. Using alanine-scanning mutagenesis, we identified two clusters of residues on opposing faces of the 4HB domain that were required for the 4HB domain to kill cells. The integrity of one cluster was essential for membrane localization, while MLKL mutations in the other cluster translocated to membranes but failed to kill; this demonstrates that membrane localization is necessary, but insufficient, to induce cell death. Finally, we identified a small molecule that binds the nucleotide binding site within the MLKL pseudokinase domain and retards MLKL translocation to membranes thereby preventing necroptosis.
SYM-28-01
RESPONSE OF COTTON BOLLWORM TO GOSSYPOL
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Plant produces secondary metabolites to resist or repel herbivores  
attack; on the other side, insects have evolved defense mechanisms  
that are able to convert or detoxify these phytochemicals. In cotton,  
gossypol and related sesquiterpene aldehydes are highly accumulated  
throughout the plant, and are toxic to insects and monogastric animals.  
However, cotton bollworm (Helicoverpa armigera) shows tolerance to  
gossypol. We identified several bollworm cytochrome P450 genes that  
play a key role in the bollworm adaptation to gossypol. When suppressed by  
plant-mediated insect RNAi technology, dramatically retarded larvae growth was observed  
when fed on transgenic cotton plants. More than tolerance, bollworm also  
utilizes gossypol to elevate their detoxification enzymes and increase  
their adaption to other toxins. Larvae pre-treated with gossypol  
showed increased tolerance to other toxic secondary metabolites and  
insecticide, including deltamethrin, a widely used pyrethroid insecticide  
in cotton field. Microarray analysis revealed gossypol-induced P450s  
exhibited high divergence and at least five of them contributed to  
bollworm tolerance to deltamethrin. Knocking down one of them,  
cytochrome P450 genes that play a key role in the bollworm adaptation  
to gossypol, has been shown to inhibit feeding, oviposition and  
larval survival. These data demonstrate that generalist insects can  
utilize gossypol to elevate their detoxification enzymes and increase  
their adaption to other toxins. Larvae pre-treated with gossypol  
showed increased tolerance to other toxic secondary metabolites and  
insecticide, including deltamethrin, a widely used pyrethroid insecticide  
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exhibited high divergence and at least five of them contributed to  
bollworm tolerance to deltamethrin. Knocking down one of them,  
cytochrome P450 genes that play a key role in the bollworm adaptation  
to gossypol, has been shown to inhibit feeding, oviposition and  
larval survival. These data demonstrate that generalist insects can  
utilize gossypol to elevate their detoxification enzymes and increase  
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showed increased tolerance to other toxic secondary metabolites and  

SYM-28-02
THE MULTINUCLEATE GENOME OF A SINGLE  
RHIZOCTONIA SOLANI AG8 ISOLATE POSSESSES A  
HIGHER LEVEL OF VARIATION THAN POPULATIONS OF  
HAPLOID PATHOGENS
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and Singh K.B.1,2  
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Rhizoctonia solani is a soil-borne basidiomycete fungus with a  
 necrotrophic lifestyle which is classified into sixteen reproductively  
 incompatible anastomosis groups (AGs). One of these, AG8, is a  
 devastating pathogen causing bare patch of cereals, brassicas and  
 legumes. R. solani is a multi-nucleate heterokaryon containing  
 significant heterozygosity within a single cell. A high quality genome  
 assembly of R. solani AG8 including 13,964 manually curated genes was  
 produced and included RNAseq and proteogenomic support (Hare et al.,  
PLoS Genetics 2014). We observed SNP-level “hypermutation” of CpG  
dinucleotides to ThpG between nucleic, with similarities to repeat-induced  
 point mutation (RIP). Unlike mononuclear fungi of the Pezizomycotina,  
gene-encoding regions were widely affected along with repetitive DNA.  
R. solani was observed to contain higher levels of diversity within its  
multiple nuclei to that held within entire populations of haploid fungal  
 pathogens. Comparative analyses with 197 phytopathogenic taxa,  
 known pathogenicity genes and other R. solani isolates predicted  
 biological processes relevant to AG8 and 308 proteins with effector- 
 like characteristics. Effector-like proteins have elevated ratios of non-  
synonymous to synonymous mutations (dN/dS), suggesting they may  
 be under diversifying selection. The moderate resistance to AG8 in  
Medicago truncatula is dependent on ethylene signalling which can  
 be manipulated to give higher resistance. However, the strong resistance  
 in Arabidopsis is independent of common defence signalling pathways  
 but depends on reactive oxygen species production, both from the  
 mitochondrial electron transport chain and RBOHs. These findings  
suggest diverse pathways may be involved in responses to this broad  
 host range pathogen in various plants.

SYM-28-03
FUNGAL CELL WALLS ARE DYNAMIC STRUCTURES  
 THAT CAN ENHANCE CELL SURVIVAL IN THE  
 PRESENCE OF ANTIFUNGAL MOLECULES
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 Melbourne Victoria.

Fungal cell walls are central to the battle between fungal pathogens and  
 innate immunity systems in both plants and animals. Innate immunity  
 molecules work by exploiting differences between the host and the  
 pathogen. In the case of bacteria there are a variety of differences  
 between the prokaryotic bacteria and the eukaryotic host that can  
 exploited in defence. However, fungi are eukaryotic and as such the  
 differences are minimal. One of the major differences between fungi  
 and host organisms is the fungal cell wall. Over the course of evolution  
 there has been a perpetual tug-of-war between pathogens and hosts.  
 That is, specific recognition of fungal cell walls by the host stimulates  
 the host’s innate immunity response including the production of antifungal  
 molecules. On the other hand the fungal cell wall acts as a defence  
 barrier that can physically block entry of the host’s innate immunity  
molecules and senses changes induced by these proteins that in turn  
stimulate a defence response from the fungus. We have examined the  
 interaction between various AMPs and components of the fungal cell  
 wall to examine how AMPs transit this barrier. We have also discovered  
 that AMPs trigger changes in the cell wall that protect the cell against  
 the deleterious effects of antifungal molecules.

SYM-28-04
MTCEP1 PEPTIDE REGULATES ROOT ARCHITECTURE  
 AND NODULATION IN THE MODEL LEGUME  
 MEDICAGO TRUNCATULA
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 Medicine, Biology and Environment, The Australian National  
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Small regulatory peptides are involved in the regulation of plant  
 development including root architecture. Recently, we examined the role  
 of a CEP (C-terminally encoded peptide) gene in Medicago truncatula  
called MEDICAGO TRUNCATULA CEP1 (MtCEP1) which is one of  
11 CEP genes. The MtCEP1 gene is predicted to encode two 15 amino  
 acid peptides with post translational modifications. Limited nitrogen  
 which modulates lateral root and nodule formation was shown to up-  
 regulate MtCEP1 expression in the roots. Further studies on MtCEP1  
 by overexpressing the peptide-encoding gene or applying the synthetic  
 MtCEP1 peptides to wild-type roots resulted in three root phenotypes:  
 (1) inhibition of lateral roots, (2) enhancement of nodulation competency  
 and (3) the formation of periodic circumferential cell proliferation (CCP)  
sites. CCP sites typically show increased periclinal and anticlinal  
cell divisions. Interestingly, lateral root and nodules could be formed  
at these CCP sites under certain conditions. Another phenotype of  
 excess MtCEP1 is the enhancement of nodulation competency which  
 includes a wider nodulation zone and partial nitrate insensitivity. Even  
at an inhibitory nitrate level of 25 mM, higher number of nodules could  
 still be formed in the presence of MtCEP1 peptide. For the lateral root  
 inhibition, closer examination showed that excess MtCEP1 peptide is  
 likely affecting the developmental programming of lateral roots prior  
to their initiation. Therefore, we hypothesize that MtCEP1 is an important  
 signaling component for modulating root architecture and nodulation in  
 Medicago truncatula.
SYM-28-05
PROTEOMIC ANALYSIS OF THE SOYBEAN SYMBIOSOME IDENTIFIES NEW SYMBIOTIC PROTEINS

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Symbiotic nitrogen fixation in legumes is characterised by the formation of a novel root organ called the nodule, within which unique organelle-like structures termed symbiosomes develop. Symbiosomes contain rhizobia which are surrounded by a membrane of plant origin termed the symbiosome membrane (SM). In this study, SM and symbiosome space (SS) fractions were isolated from nitrogen fixing soybean root nodules and analysed using non-gel proteomic techniques. Bicarbonate stripping and chloroform-methanol extractions of isolated SM were used to enrich for hydrophobic integral membrane proteins. One-hundred and seventy-two proteins were identified as components of the SM, with an additional fifteen proteins identified from peripheral membrane and SS protein fractions. Proteins involved in a range of cellular processes such as metabolism, protein folding and targeting, signalling and transport were identified. These included a number of proteins previously localised to the SM, such as nodulin 26, an aquaglyceroporin. Transport classes identified include sulphate, calcium, peptide/dicarboxylate, nitrate and metal ion transporters. Several putative transporter candidates have been characterised further by gene expression analysis and reduction studies and GFP-tagged protein localisation. The transport function of candidates was investigated using yeast complementation assays, with peptide and amino acid transport capabilities detected. This study provides a comprehensive analysis of the protein content of the soybean SM, and will be an invaluable tool for future studies on the legume-rhizobia symbiosis.

SYM-29-02
IMPROVING THE SALT TOLERANCE OF BREAD AND DURUM WHEAT

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Soil salinity occurs in all semi-arid regions of the world, but these are crucial in providing sufficient food to feed the growing populations. Salinity is widespread in southern Australia, and limits crop production in many areas. Increases in plant salt tolerance will lift yield in these salty soils. Mechanisms of the more salt-tolerant crops such as barley are osmotic adjustment using Na and Cl as energy-efficient osmotica, safely compartmentalised in the vacuole. The more sensitive crops cannot store high salt concentrations in the vacuoles, so their roots keep the salt out of the leaves by excluding salt from the transpiration stream. This comes with an energy cost, but it avoids salt toxicity. In an ancestral wheat species, we identified two novel transporters of the HKT type that can retrieve salt from the transpiration stream and prevent it flowing to the leaves. Both genes are expressed only in xylem parenchyma cells. Yeast and Xenopus oocyte expression systems showed them to be Na-selective transporters. These genes were introduced into durum wheat by conventional breeding and tested on farmers’ fields. They lowered the Na concentration in the leaves and one gene, HKT1;5-A was able to increase the yield of durum wheat in saline soils by 25%. This gene is similar to HKT1;5-D that is present in bread wheat but not durum wheat. Silencing HKT1;5-D in bread wheat resulted in higher Na accumulation in the leaf blade. This explains the higher salt tolerance of bread over durum wheat, and provides an opportunity to increase the salt tolerance of bread wheat further.

SYM-29-03
MOLECULAR BREEDING FOR ABIOTIC STRESS TOLERANCE AND IMPROVED NUTRIENT-USE EFFICIENCY

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Crop production is constraint by numerous abiotic stresses, such as drought, heat, salinity, and unfavourable soil properties, causing significant reduction in yield. Developing crops with abiotic stress tolerance can therefore contribute significantly to closing the yield gap thereby increasing crop productivity and food security. Mapping of quantitative trait loci (QTL) and cloning of major genes has proven a valid approach providing access to an increasing number of high-value genes which can be used for marker-assisted breeding of tolerant varieties. This approach has been successfully applied for e.g., the rice submergence-tolerance gene SUB1A and the phosphorus-starvation tolerance gene PSTOL1. In wheat, barley and other crops, genes for tolerance to salinity, boron and aluminium toxicity have already been identified, as well as promising QTL for heat and drought tolerance. These examples demonstrate the potential of molecular technologies for the development of crops that are more resilient and productive under unfavourable growth conditions, as prevalent in Australia. Resilient crops will also contribute to improved fertilizer-use efficiency. However, studies in wheat recently revealed that significant genotypic differences in nitrogen (N) use efficiency exist in Australian wheat germplasm, with the majority of the tested varieties showing little yield increase with additional N applied. This might be due to a prevalent selection of breeders for stress tolerance rather than for high yield potential. A combination of forward and reverse genetic approaches and a multi-disciplinary approach towards molecular breeding is needed to ensure relevance of research and impact.
SYM-29-04

INVESTIGATION OF THE REGULATORY MECHANISMS CONTROLLING CYANOGENESIS IN FORAGE SORGHUM

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Forage sorghum is an important source of animal feed, however, sorghum produces the potentially toxic cyanogenic glycoside, dhurrin. Upon tissue disruption this leads to the release of hydrogen cyanide (HCN). HCN concentration increases with drought and high nitrogen levels and Australia loses approximately $20 million a year due to fear of toxicity to cattle. At Monash University, in collaboration with Pacific Seeds and the University of Copenhagen, we have developed sorghum lines with altered levels of cyanogenic potential. The synthesis of dhurrin involves two cytochrome P450s (CYP79A1, CYP71E1) and sorghum lines with altered levels of cyanogenic potential. The synthesis of dhurrin involves two cytochrome P450s (CYP79A1, CYP71E1) and a UDP-glucosyltransferase (UGT85B1). CYP79A1 is considered rate limiting and was targeted in the EMS mutation program resulting in the identification of the mutant, adult cyanide deficient class 1 (acdc1). The mutant acdc1 contains high levels of dhurrin in the leaves of young seedlings but negligible levels in adult leaves. This is an advantage for agricultural production as the plants are protected from herbivory when very young but then become non-toxic and suitable for animal fodder as they develop. In addition, acdc1 mutants grow rapidly resulting in plants with high biomass. No mutations in the CYP79A1 structural gene sequence have been detected and, in conjunction with the observed phenotype, this suggests that acdc1 may be a regulatory mutant. To analyse this further, 1.2kb of the acdc1 CYP79A1 promoter has been sequenced and we have identified a putative EMS mutation, which may be responsible for the phenotypic change. Potential cis-acting regulatory elements have been identified using database analysis and we are currently investigating the mechanisms regulating cyanogenesis in the acdc1 mutants, using such techniques as gel shift assays.

SYM-29-05

GRAIN YIELD OF SALT STRESSED CHICKPEA IS IMPROVED BY SUGAR SUPPLY AT THE REPRODUCTIVE STAGE

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Reproductive processes of chickpea (Cicer arietinum L.) are particularly salt sensitive. Reproductive failure of chickpea under salt stress could be due to direct ion toxicity or limitations of photosynthate supply to reproductive tissues. This experiment was designed to test whether increased sugar supply could overcome the reproductive failure of salt-stressed chickpea. Rupali, a particularly salt sensitive genotype, was grown in control conditions or salt stress (30 mM NaCl) for 100 days in nutrient solution culture. At the time of flowering, some plants were infused with sucrose solution (0.44 M) into the stem, infused with water only, or maintained without any infusion. Both vegetative growth and reproductive processes were severely reduced by the 30 mM NaCl treatment. Sucrose or water infusion in plants without salt stress had no effects on growth and yield. Under salt stress, non-infused and water-infused plants did not differ. However for salt-stressed plants, sucrose infusion increased dry mass per plant (3 fold) as compared with non-infused plants. Sugar infusion also increased the number of pods per plant by 4 fold and the number of seeds and seed yield by 6 fold and 10 fold, respectively, but seed yield still remained lower than for plants in non-saline conditions. Individual seed size was reduced under salt stress, but sucrose infusion did not influence seed size. We conclude that insufficient photosynthate availability limits yield in salt-stressed chickpea.

SYM-30-01

MOLECULAR REGULATION OF STEM CELL QUIESCENCE

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Many adult stem cells persist in a non-cycling, quiescent state. We have previously demonstrated that the quiescent state of adult muscle stem cells (MuSCs) is actively maintained by Notch signaling and that a transcriptional regulatory pathway involving the microRNA miR489. In recent studies, we have explored the dynamics of the quiescent state. Normally, nearly all MuSCs exist in a conventional quiescent state, G0. However, in response to a distant injury, for example an injury to a muscle in a different limb or injury to skin, MuSCs of a limb muscle are conveyed through diverse stem cell compartments of the organism. For many weeks after the inciting stimulus, we propose that the G0 state maintains the appropriate properties for weeks many weeks after the inciting stimulus. We propose that the G0 state represents a novel form of cellular memory in which a previous stimulus results in a long-lasting cellular response that allows stem cells to respond more rapidly and effectively to subsequent challenges for tissue homeostasis and repair. Furthermore, these data demonstrate that systemic signals from tissue injury communicate widely to diverse stem cell compartments of the organism.

SYM-30-02

METABOLIC REPROGRAMMING IN SKELETAL MUSCLE STEM CELLS

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Through advances in cancer and stem cell biology, it has become apparent that changes in cellular metabolism play a large role in the regulation of stem cell function - a process termed 'metabolic reprogramming'. The first evidence of metabolic reprogramming was provided by Otto Warburg, who found that tumour cells preferentially utilized the glycolytic pathway even in the presence of oxygen. This process was referred to as aerobic glycolysis, and later the “Warburg effect”. Recently, a similar process of metabolic reprogramming has been identified in stem cell populations, including the population of skeletal muscle stem cells (MuSCs) responsible for the large regenerative capacity of this tissue (Ryall, FEBS J 280:4004-13, 2013). Using the myogenic C2C12 cell line we have developed and refined a technique of metabolic reprogramming; a process that involves forcing C2C12 cells to utilize oxidative phosphorylation rather than glycolysis (via incubation in a reduced glucose or galactose based growth media). Following a single passage (24 hrs), metabolically reprogrammed C2C12 cells exhibited an increased rate of oxygen consumption and reduced extracellular acidification; this was associated with an increase in the rate of cellular proliferation. Whole transcriptome sequencing of reprogrammed cells revealed a decrease in the specification and commitment of these cells (including downregulation of Myod1 and Myf5). Furthermore, western immunoblotting confirmed a decrease in the protein levels of several myogenic commitment factors following reprogramming. These exciting results strongly support a role for cellular metabolism in regulating myogenic cell identity.
SYM-30-03
MYELIN REGENERATION BY PRECURSOR CELLS IN THE ADULT CENTRAL NERVOUS SYSTEM
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Parenchymal oligodendrocyte progenitor cells (pOPCs) are considered the principal cell type responsible for oligodendrogenesis and remyelination in demyelinating diseases. Recent studies have demonstrated that neural precursor cells (NPCs) from the adult subventricular zone (SVZ) can also generate new oligodendrocytes following demyelination. However, the relative contribution of NPCs versus pOPCs to remyelination is unknown. We used in vivo genetic fate-mapping to directly assess the behaviour of each progenitor type within the corpus callosi (CC) of mice subjected to cuprizone-induced demyelination. Nestin-CreERT2 and Pdgfra-CreERT2 transgenic mice were crossed with fluorescent Cre reporter strains to map the fate of NPCs and OPCs respectively. In cuprizone-challenged mice, substantial numbers of NPCs migrated into the demyelinated CC and contributed to oligodendrogenesis. This capacity was most prominent in rostral regions adjacent to the SVZ where NPC-derived oligodendrocytes outnumbered those generated from pOPCs by 4.6-fold, indicating that NPCs have a significant competitive advantage over pOPCs for oligodendrogenesis in this region. Sixty-two percent of all nodes of Ranvier in the rostral regions were flanked by at least one paranode generated from an NPC-derived oligodendrocyte. Moreover, myelin thickness in regions subject to significant NPC-derived oligodendrogenesis was equivalent to unchallenged controls indicating that these cells make a major contribution to restoration of normal myelin caliber. We also demonstrated that a reduced efficiency of remyelination in the caudal CC was associated with impaired maturation of oligodendrogenic NPCs. Collectively, our data define a major role for NPCs in remyelination, identifying them as a key target for enhancing myelin repair in demyelinating diseases.

SYM-30-04
YAP ACTIVATES BETACATENIN IN MURINE EPIDERMAL STEM/PROGENITOR CELL PROLIFERATION
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Skin cancer accounts for 80% of newly diagnosed cancers in Australia. Oncoprotein Yes-associated protein (YAP) is a pivotal and highly conserved regulator of stem cells and organ size that is active in human cancer. We have recently generated a transgenic mouse model that expresses a constitutively active form of YAP protein mutant YAP2-5SA-AC in the basal epidermal cells, and survives postnatal life. YAP2-5SA-AC mice display hyperpigmentation, and a dramatic expansion of epidermal stem/progenitor cell populations in the interfollicular epidermis and in the hair follicle bulge. Wnt/β-catenin signaling is another pivotal regulator of epidermal and melanocyte stem/progenitor cell proliferation. We found that YAP and β-catenin co-localize in epidermal bulge stem/progenitor cells, and found evidence suggesting that YAP may activate β-catenin in bulge stem cell proliferation. Interestingly, we also found that YAP and β-catenin co-localize in the nuclei of human skin cancers. Taken together, this work supports the existence of a positive regulatory interaction between YAP and β-catenin in the regulation of epidermal stem/progenitor cell proliferation during normal skin homeostasis, which may be disrupted in the etiology of skin cancer.

SYM-30-05
THE UBIQUITIN SPECIFIC PROTEASE, USP9X, IS REQUIRED FOR THE CELLULAR PROLIFERATION AND STABILISATION OF MTORC1 COMPONENTS P-MTOR(SER2448), IN NEURAL STEM CELLS (RENCILLS VM) IN VITRO AND IN VIVO
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USP9x is a deubiquitlating enzyme that is highly expressed in neural progenitors in the developing murine brain. Its deletion from neural progenitors in the developing mouse brain leads to embryonic lethality implicating a requirement for progenitor maintenance and growth as it is known that slight increases of USP9x enhance self-renewal in vitro. Deletion of USP9x from the forebrain alone, has resulted in reduced hippocampal size which may implicate USP9x as a contributor in neural structure growth and proliferation. To date, the role of USP9x in cell cycle regulation of neural progenitors is not fully understood. Preliminary evidence from USP9x-/y neurospheres suggested reduction in sphere size and number, with no change in sphere forming capacity. To determine if USP9x has a role in cellular proliferation, we depleted USP9x from immortalised neural stem cell line, Rencells VM. We observed a reduction in adherent cellular density, reduced cellular proliferation and an accumulation of cells in the G0/G1 phase. To determine if cells were arresting at the G1-S phase transition, we investigated the status of cell cycle proteins involved at this phase and showed a reduction in phosphorylated retinoblastoma protein, pRbS780, whilst other cell cycle proteins levels remained unchanged. We also show that after EGF/FGF stimulation there is reduced mTORC1 activity, as measured by phospho-S6 ser235/236, in USP9x-depleted Rencells VM. We further demonstrated this was possibly due to the reduction of mTORC1 p-mtor (Ser2448) and Raptor in vitro and confirmed mTORC1 signalling was perturbed, with reduced p-mTOR (ser2448) expression in E17.5 USP9x-/y neural progenitors. This potentially indicates that USP9x may stabilise p-mTOR (ser2448) signalling in neural stem cells.

SYM-31-01
STRUCTURAL BASIS FOR IRON PIRACY BY PATHOGENIC NEISSERIA
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Neisseria are obligate human pathogens causing bacterial meningitis, septicemia, and gonococcal. Neisseria require iron for survival and can extract it directly from human transferrin for transport across the outer membrane. The transport system consists of TbpA, an integral outer membrane protein, and TbpB, a co-receptor attached to the cell surface; both proteins are potentially important vaccine and therapeutic targets. Two key questions driving Neisseria research are: 1) how human transferrin is specifically targeted, and 2) how the bacteria liberate iron from transferrin at neutral pH. To address them, we solved crystal structures of the TbpA-transferrin complex and of the corresponding co-receptor TbpB. We characterized the TbpB-transferrin complex by small angle X-ray scattering and the TbpA-TbpB-transferrin complex by electron microscopy. Collectively, our studies provide a rational basis for the specificity of TbpA for human transferrin, show how TbpB promotes iron release from transferrin, and elucidate how TbpB facilitates this process.
SYM-31-02

A RECEPTOR FOR HUMAN HAEMOGLOBIN ON THE SURFACE OF PATHOGENIC STAPHYLOCOCCUS AUREUS

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Iron has unique properties that make it essential to eukaryotic and bacterial cells alike. Pathogens must acquire the iron they need from the infected host and many bacteria express cell-surface proteins dedicated to capturing iron from host proteins. We have studied the mechanisms by which the human pathogen, Staphylococcus aureus, captures human haemoglobin and removes the iron-containing haem group. These functions are achieved by a dedicated haemoglobin receptor that is embedded in the cell wall of the Gram-positive S. aureus. The haemoglobin receptor forms the tip of a haem-relay pathway that shuttles haem across the cell wall and membrane, into the bacterial cytoplasm. This pathway is broadly conserved across Gram-positive bacterial pathogens, although the mechanisms that target host haem-proteins at the top of the pathway may be diverse.

SYM-31-03

A NOVEL 'NUCLEOTIDE-BOUND OUTWARD-OCCULDED' CONFORMATION OF AN ANTIBACTERIAL PEPTIDE ABC TRANSPORTER

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Specific Enterobacteriaceae produce antimicrobial peptides under nutrient limited conditions. Antimicrobial peptide microcin J25 (MccJ25) has a unique lasso topology. MccJ25 expression is upregulated under iron limited condition and imported by an iron importer (FhuA), consequently killing the bacterium. Providing MccJ25 producing cells with an advantage over other cells under nutrient limited conditions. Mature toxic MccJ25 secretion and self-immunity is conferred by an ATP-binding cassette (ABC) exporter McjD. The x-ray crystal structure of McjD from E. coli was determined at 2.7 Å resolution, the first structure of an antibacterial peptide ABC transporter. McjD was seen in a new conformation of nucleotide-bound outward-occluded, defining a clear ligand cavity. The new conformation shows similarities to both inward-open McbA and outward-open Sav1866 structures. An almost rigid rotation of TM1 and TM2 towards the equivalent TMs of the opposite monomer forms the outward-occluded conformation. This conformation has been confirmed by cysteine cross-linking studies. Therefore, we propose that the outward-occluded state represents an intermediate conformation between the outward-open and inward-open conformation of ABC exporters. Biochemical analyses also demonstrate McjD-dependent immunity to MccJ25 through efflux of the peptide. McjD can directly bind MccJ25, and displays a basal ATPase activity that is stimulated by MccJ25 in both detergent solution and proteoliposomes.

SYM-31-04

CHARACTERISING THE STRUCTURAL DYNAMICS OF PNEUMOCOCCAL SURFACE ADHESIN A BY MD SIMULATIONS AND EPR SPECTROSCOPY

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Pneumococcal disease is a leading cause of death in the elderly and children under 5, particularly amongst Aboriginal and Torres Strait Islander communities. Pneumococcal surface adhesin A (PsaA) is membrane-tethered protein located on the cell-surface of all known pneumococcal serotypes. PsaA is a major virulence factor that is essential for host colonization and mediating disease. PsaA has a demonstrated dual role as a metal binding protein that delivers manganese (Mn2+) to the Psac permease domain of its cognate ABC importer; and as a surface adhesin that binds to the E-cadherin receptor of human nasopharyngeal cells. PsaA is a promising target for the development of serotype-independent vaccines and antimicrobial therapeutics for the treatment of pneumococcal disease. The recent crystal structures of PsaA in the metal-free (apo) and metal-bound conformations have laid the groundwork for rational design of antimicrobial drugs that target PsaA. These crystallographic structures represent closely related conformations of PsaA, but do not provide information about the range of conformations that PsaA can adopt in solution, or the dynamic transitions that may occur. To address this issue we carried out molecular dynamics (MD) simulations of apo PsaA in conjunction with continuous-wave electron paramagnetic resonance (CW-EPR) spectroscopy experiments. From the combined results, the overall conformational dynamics of the protein and the relative mobility and solvent accessibility of each domain was determined. A comparison of the data from MD simulations and CW-EPR and an analysis of the structural dynamics of PsaA with respect to the proposed metal-binding mechanism will be presented.

SYM-31-05

A MORTISE TENON JOINT IN THE TRANSMEMBRANE DOMAIN MODULATES AUTOTRANSporter ASSEMBLY INTO BACTERIAL OUTER MEMBRANES

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Bacterial autotransporters comprise a 12-stranded membrane-embedded β-barrel domain, which must be folded in a process that entraps segments of an N-terminal passenger domain. This first stage of autotransporter folding determines whether subsequent translocation can deliver the N terminal domain to its functional form on the bacterial cell surface. Here, paired glycine aromatic mortise and tenon motifs are shown to join neighbouring β-strands in the C terminal barrel domain, and mutations within these motifs slow the rate and extent of passenger domain translocation to the surface of bacterial cells. In line with this, biophysical studies of the autotransporter Pet show that the conserved residues significantly quicken completion of the folding reaction and promote stability of the autotransporter barrel domain. Comparative genomics demonstrate conservation of glycine aromatic residue pairings through evolution as a previously unrecognized feature of all autotransporter proteins.
INTEGRATIVE GENOMICS APPLIED TO HUMAN COMPLEX DISEASES: A STUDY OF CORONARY ARTERY DISEASE

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Global efforts in producing detailed tissue-level omics data are expected to produce an unprecedented wealth of information on gene interaction and regulation. Simultaneously, network-based approaches are evolving to fulfill the increasing need for statistical analysis, visualization and ultimately biological interpretation of the findings. Coronary artery disease (CAD) is an important example of a complex disease, where integration of molecular resources can be useful. In particular, the majority of the heritability remains unexplained, despite large-scale genome-wide association studies (GWAS). Integrating functional genomic data with the GWAS may facilitate the identification of novel biological processes and genes involved in CAD, as well as clarify the causal relationships of established processes. We combined 14 GWAS from the CARDIoGRAM Consortium and two additional GWAS from the Ottawa Heart Institute (25,491 cases and 66,819 controls) with 1) genetics of gene expression studies of CAD-relevant tissues in humans, 2) metabolic and signaling pathways from public databases, and 3) data-driven, tissue-specific gene networks from multiple human and mouse experiments. We detected CAD-associated gene networks of lipid metabolism, coagulation, immunity, and additional networks with no clear functional annotation, and also revealed key driver genes for each CAD network based on the topology of the gene regulatory networks. In particular, we found a gene network involved in antigen processing to be strongly associated with CAD. The key driver genes of this network included glyoxalase I (GLO1) and peptidylprolyl isomerase I (PPIF), which we verified as regulatory by siRNA experiments in human aortic endothelial cells. Our results suggest genetic influences on a diverse set of both known and novel biological processes that contribute to CAD risk. The key driver genes for these networks highlight potential novel targets for further mechanistic studies and therapeutic interventions.

SYM-32-01

SYM-32-03

POPULATION METAGENOMICS OF A DEVELOPING INFANT GUT MICROBIOME

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The human body plays host to a complex microbial ecosystem, the development of which begins at or even before the time of birth. Routine monitoring of the development of microbial ecosystems in newborns (or other environments) using metagenomic methods is currently extremely challenging and expensive. I will describe some recent technological advances that could enable routine sequencing and computational analysis of hundreds of metagenomes, and demonstrate their application on samples taken from fecal microbiome of an infant during the first months of life. In this study forty-five samples were subjected to transposon-catalyzed illumina library prep and metagenomic sequencing on a HiSeq 2000 instrument. The resulting data was subjected to analysis of microbial community structure using a new approach called phylogenetic Edge Principal Component Analysis (Edge PCA) that can identify which lineages in a phylogeny explain the majority of variation among the samples. I will also describe population genomics of Bacteroides, one of the dominant members of the gut microbial community. Major life events in the infant appear to be associated with dramatic change in the gut microbiota.

SYM-32-04

GENOMIC STRATEGIES AND FUNCTIONAL INSIGHTS TO NOVEL EYE DISEASE GENES

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Next-generation sequencing (NGS) including whole exome (WES) and whole genome sequencing (WGS) provide unparalleled opportunities for novel gene discovery. However, functional validation of the candidate disease genes is a major bottleneck in the course of knowledge translation. This study presents a pipeline approach to elucidate the functional role of disease-causing genes using the example of novel candidates in eye disease. Four novel candidate disease genes were identified using WES and WGS in patients with eye anterior segment and retinal diseases. WES in extended patient cohorts identified additional likely pathogenic variants. The functional domains in the candidate genes predict roles in Wnt signalling, cell adhesion and golgi function. To evaluate this broad spectrum of functions and disease mechanisms, we developed a pipeline for functional validation including: expression analyses of cell-based assays using shRNA and predicted pathogenic variant transfection, morpholino studies in zebrafish and mouse model analyses. We have successfully characterized two new Wnt signalling factors contributing to human disease, one affecting iris development and the other eye size. We have also identified a novel factor regulating cytoskeletal organization and epithelial cell polarity, and are further investigating the putative golgi protein. Our pipeline approach using cell-based assays provides a rapid experimental system to test function of novel candidate genes identified from NGS discovery projects. Expeditious results were obtained from zebrafish analyses and provided impetus for mouse studies for further mechanistic understanding of the roles of the encoded proteins.
results in developmental defects. Research is focused on genetic or both in combination. Such disruption to signal transduction can be disrupted by genetic and environmental factors, in a way that impacts on cell behaviour and morphogenesis. Signal receptor and a cascade of intracellular events alters gene expression well defined and considered to be hard wired as a ligand activates its of development and in many cellular contexts. These pathways are transduction pathways are used to shape the embryo at many stages.

**SYM-32-05**

**RBP ATLAS: AN EXPLORATION OF INTERACTIONS BETWEEN MRNA AND PROTEINS AND THEIR IMPACT ON CARDIOMYOCYTE BIOLOGY**

Liao Y.1, Fischer B.2, Castello A.2, Yang H.1, Foehr S.2, Leicht S.2, Rasdilshav H.2, Jeroen K.2, Henze M.1 and Preiss T.1

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1 JCMSR, Australian National University, Canberra, Australia 2 EMBL, Heidelberg, Germany RNA-binding proteins (RBPs) control all aspects of RNA fate, often by organizing multiple functionally related RNAs into “post-transcriptional-operons”. RBP functional defects furthermore underlie a broad spectrum of human pathologies. How such RBP networks operate in the heart is poorly understood. We chose to investigate this in murine HL-1 cardiomyocyte cell line, which maintains contraction ability and other differentiated cardiac properties. Deploying a recently developed “mRNA interactome capture” method (Castello et al., Cell. 149:1395), we have now identified ~1100 proteins as the first cardiomyocyte “mRNA interactome”. Domain features and gene ontology enrichment broadly validate the sensitivity and specificity of the capture. Of direct relevance to cardiac biology, ~180 RBP genes are associated with heart disease (genecards.org). ~20 are differentially regulated during cardiac reprogramming, most of these have no established RNA links. Notably, ~80 cardiomyocyte RBPs are enzymes of intermediary metabolism, which otherwise perform housekeeping metabolic functions, they are enriched for mitochondrial energy metabolism. Furthermore, in an “RBDbmap” validation experiment, 320 proteins were validated by uncoupling their RNA binding regions, including a wide range of classical/non-classical RNA-binding domains. A highlight of HL-1 RBDbmap is illustrating RNA binding nature for domains such as nucleotide binding (e.g. ATP, NAD+), iron-sulfur cluster binding, and those harboring gene mutations of mitochondrial diseases. Our work links cellular metabolism and gene expression through RNA-binding by metabolic enzymes. Ongoing work is focused on characterizing RNA regulatory functions of mitochondrial enzymes in TCAC/OXPHOS, and defining how their dysregulations may be part of diseases processes.

**SYM-33-01**

**REGULATION OF SIGNAL TRANSDUCTION BY CAVEOLAE**

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Caveolae are abundant cell-surface pits that have been implicated in lipid regulation, signal transduction, and endocytosis. Caveolins, the major membrane proteins of caveolae, play a crucial role in the formation of caveolae. Mutations in caveolins are associated with breast cancer and with a number of metabolic diseases. We have studied how caveolin-lipid interactions generate the unique architecture of the caveolar domain by development of a model prokaryotic system for caveola formation. Our studies have identified a new family of coat proteins, termed cavins, that regulate caveola formation in vertebrate cells. Study of the cavins and their dynamics of caveola association/dissociation provides new insights into the role of caveolae. In response to membrane stress caveolae flatten with release of cavins into the cytoplasm. In cell culture, in mammalian muscle explants, and in zebrafish embryos we demonstrate that caveolae can protect cells against mechanical damage. The molecular mechanisms whereby caveolae exert control over cellular signalling have to date remained elusive. We have therefore explored the role caveolae play in modulating Ras signalling. Lipidomic and gene array analyses revealed that caveolin-1 (CAV1) deficiency results in altered cellular lipid composition, and plasma membrane (PM) phosphatidylserine distribution. These changes correlated with increased K-Ras expression and extensive isoform-specific perturbation of Ras spatial organization: in CAV1 deficient cells K-RasG12V nanoclustering and MAPK activation were enhanced, whereas GTP-dependent lateral segregation of H-Ras was abolished resulting in compromised signal output from H-RasG12V nanoclusters. In addition to these effects on plasma membrane lipid organization, we have identified interactions between cavins and cytoplasmic targets when cavins are released from the plasma membrane in response to membrane stress. These studies highlight the role of caveolae in specific signal transduction pathways and provide new insights into the role of caveolae in disease.

**SYM-33-02**

**GENETIC AND ENVIRONMENTAL FACTORS AFFECT SIGNAL TRANSDUCTION DURING MOUSE EMBRYOGENESIS**

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Embryogenesis is all about Signaling in Space & Time. Signal transduction pathways are used to shape the embryo at many stages of development and in many cellular contexts. These pathways are well defined and considered to be hard wired as a ligand activates its receptor and a cascade of intracellular events alters gene expression in a way that impacts on cell behaviour and morphogenesis. Signal transduction can be disrupted by genetic and environmental factors, or both in combination. Such disruption to signal transduction results in developmental defects. Research is focused on genetic and environmental factors that disrupt signaling in the context of somitogenesis and vertebral column formation.

**SYM-33-03**

**RECONSTITUTION OF PROTEIN CLUSTERS THAT ORGANIZE T CELL RECEPTOR PHOSPHORYLATION SIGNALING NETWORK REGULATED BOTH POSITIVELY AND NEGATIVELY BY PHOSPHATASE CD45**

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In many different signaling systems, the clustering of signal proteins has been reported as a key regulatory mechanism to induce signal transduction. In T lymphocyte membranes, T cell receptor and other proteins associated with plasma membranes form submicron-sized clusters to induce the signaling, upon the activation of receptors with their ligands. Studies using advanced imaging technologies have revealed the structures and compositions of those signal protein clusters. However, how the T cell signaling is regulated in those clusters has not been well understood. In order to quantitatively understand how the network of signal transduction in T cell is modulated by protein clustering, we developed a reconstitution system that consists of purified signal proteins and supported lipid bilayers as a mimic of cell membrane. In this system, we could artificially cluster proteins on bilayers, and analyze biochemical reactions in the clusters by fluorescence microscopy. We focused on the signal network that consists of T cell receptor (TCR), kinase Lck, and phosphatase CD45, and found that differential clustering of Lck result in distinct regulations of TCR phosphorylation by CD45. In the protein clusters that contain diffusive Lck, TCR was regulated only negatively by CD45, while in clusters that contain immobile Lck, the TCR phosphorylation was also positively regulated by CD45. Such dual positive/negative roles of CD45 have been known from genetic/functional studies of T cells. We further analyzed the Lck activities in both the reconstituted systems and in T cells, to explore more details in the regulatory mechanisms of protein clusters.
SYM-33-04

PLASTICITY IN THE FUNCTIONAL NETWORKS THAT COMPRISE AND CONTROL CELL MIGRATION

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Cell Migration is a complex, dynamic and heterogeneous phenomenon emergent over multiple spatiotemporal scales. Such heterogeneity is under-represented in the understanding of migration because common-aggregate experimental approaches impose insensitivities to natural variability. Furthermore, perturbation-based methods used to dissect such complex processes may induce systemic distortions, even given high target specificity, due to dense regulatory interdependencies, and potentially undermining functional inferences. Addressing these two common limitations, we utilize a Systems Microscopy research framework that instead leverages natural heterogeneity to reveal functional networks underlying the cell migration system, without dependence on experimental perturbations. This is enabled by imaging-based acquisition of time-resolved single cell data recording hundreds of quantitative features simultaneously defining migration system organization and behaviour, capturing macromolecular- (adhesions, F-actin, membranes) and cellular-scales (morphology, polarity, motility). We define functional relationships between organizational and behavioural features using various statistical techniques, including moving beyond canonical correlative analyses via cutting-edge Grapher causality mapping to define directionally specific causal influence networks. Extending on our recent finding that these networks are perturbation-sensitive, we now show spontaneous re-wiring of causal networks given natural heterogeneity in cell speed. Spontaneous plasticity is also observed as cells stochastically adopt interconvertible modes of migration with distinct regulatory dependencies. To further explore migration system plasticity (within several parallel studies), we progressively modulate core regulatory axes to map one- and two-dimensional landscapes of adaptation. These studies reveal specific non-linear, non-monotonic and context-dependent responses to continuous variations in: mechanical tension; protein expression; and ECM ligand density. Overall, this Systems Microscopy approach unmasks the profound and fundamental plasticity of the functional networks that comprise and control cell migration.

SYM-33-05

MULTIPLE ROLES FOR THE DROSOPHILA NETRIN RECEPTOR, FRAZZLED, IN EPITHELIAL MESENCHYMAL TRANSITIONS

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Epithelial Mesenchymal Transitions (EMTs) are important in cancer metastasis and development. We are studying an EMT-like event called wing disc eversion, whereby peripodial epithelial cells degrade the basement membrane (BM) via MMPs, lose apico-basal polarity, delocalize ECadherin and extend FActin rich protrusions during invasion and migration over the epidermis. Eversion failure leads to disrupted wing/thorax formation. In an RNAi screen for evasion genes we identified the chemotacticant netrinA. netrinA RNAi suppressed ECad dissociation, but not FActin reorganization, JNK activation (1), or BM breakdown. Several lines of evidence indicate NetrinA promotes EMT by downregulating its receptor, Frazzled (Fra); i) fra RNAi accelerates EMT, ii) netrinA RNAi increases Fra levels, iii) netrA RNAi phenotypes are suppressed by the fra deficiency Df(2R)BSC880 or fra RNAi; iv) overexpression of Frazzled inhibits evasion (1). Surprisingly, increasing Fra levels reduces evasion phenotypes but promotes FActin protrusions, presumably via the known Fra motility pathway mediating axonal pathfinding. Consistent with this, fra transgenes lacking the P3 domain (crucial for axonal guidance), induce fewer protrusions. Interestingly, fra3/4/4 mutants exhibit thoracic clefts suggesting Fra-induced motility may function during epithelial migration. To determine downstream Frazzled pathways we are utilizing mosaic fra expression and analysis of fra deletion transgenes w.r.t cellular and adult phenotypes. The Fra epithelial pathway likely acts through Moesin, since moe RNAi rescues netrA RNAi phenotypes and Fra promotes Moe phosphorylation. Fra overexpression also relocates basal pMoe and ECad in epithelial cells, and appears to reduce basal contractility suggesting Rho1 repression. We are now testing the involvement of potential Moeis kinases (e.g. SLIK, ROK, PI3K, SFKs), the RhoGAP Conundrum and Fra-interacting axon guidance factors (e.g. TRIO, Abi, Era). 1. Manhire-Health et al, 2013, Nat. Commns. 4, 2790.

SYM-34-01

DEVELOPMENT OF A ‘LEARNING AND TEACHING ACADEMIC STANDARDS STATEMENT’ FOR AGRICULTURE

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We report on the process and outcomes of a national project that has developed a Learning and Teaching Academic Standards (LTAS) Statement for the Agriculture discipline. Agricultural research and teaching relies on strong links with industry due to the applied nature of the discipline. Without these links, sustainable and profitable practice change in agricultural systems cannot be achieved. A pilot project, in 2011-2012, with academic staff from three Australian universities identified Threshold Learning Outcomes (TLOs) for the sub-discipline of Agricultural Science. The AgLTAS project provided the opportunity to validate or refute these TLOs by seeking input from a wider group of stakeholders, including industry. National consensus was sought by a process of iterative consultation with academics, students and industry stakeholders and tested across four Australian universities. We have collected qualitative and quantitative data from participants who attended a series of workshops across most Australian States and Territories and via an online survey. The need for students to attain highly developed problem solving and communication skills was highly valued. While perceived benefits to students were perceived, institutional benefits were not evident. Such outcomes have the potential to drive new opportunities and innovation in agriculture. Industry-specific (vocational) knowledge was generally regarded as attainable during on-the-job training after graduation. The next phase of the project aims to trial the Standards Statement for Agriculture by benchmarking the academic standards achieved in four universities that teach Agriculture and related disciplines, using an online Curriculum Mapping Tool.

SYM-34-02

MAPPING ASSESSMENT PRACTICES TO PROVIDE INSIGHT INTO LEARNING AND TEACHING

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It is widely accepted that assessment of learning is a critical component of education and that assessment drives/guides student learning through shaping study habits and student approaches to learning. Further, the need for competency and expertise, in regards to assessment, in the Australian academic community has increased with a heightened focus on learning outcomes, standards and employability skills. There is therefore, a critical need to both ensure and demonstrate the quality, validity and standards of assessment practices. To address this we developed a tool to map assessment practices. The assessments are mapped to a range of criteria including: assessment type, format, timing, assessors, provision of feedback, level of learning (Bloom’s taxonomy), approaches taken to planning assessment and their alignment with learning outcomes (LOs) across units, majors and degree programs. In addition the tool enables the mapping of assessment to the Australian Science Threshold Learning Outcomes (TLOs), the Biomedical Science TLOs and the ASBMB (USA) core concepts and skills. A major advantage of the mapping tool is its ability to integrate the data and provide insight into the systematic development of higher order learning and skills progression throughout a program of study. The mapping of assessment to threshold learning outcomes will provide a valuable tool for students to compare the data they provide can be used as evidence of teaching and learning and student attainment of the appropriate standards.

SYMPOSIUM

Page 66

ComBio2014 • Canberra, ACT, Australia • 28 September – 2 October, 2014
SYM-34-03

THE DEVELOPMENT OF UNDERGRADUATE SCIENCE STUDENTS’ SCIENTIFIC ARGUMENT SKILLS IN ORAL PRESENTATIONS

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The Science Threshold Learning Outcomes (TLOs) developed recently as part of the Learning and Teaching Academic Standards project, reinforce the ability to develop evidence-based, well-reasoned arguments and to clearly communicate those arguments in a variety of communication modes, are key graduate attributes (Jones, Yates & Kelder, 2011). However, in practice, specific measurement of these skills is limited, particularly in oral presentations. This study describes the initial literature-based development of a rubric for the evaluation of scientific argument in oral presentations (Toulmin, 1958; Sampson, Grooms & Walker, 2009), and the reiterative, data-driven process of refinement of that rubric. The rubric reflects the established framework for the scientific argument, by including criteria for claim, evidence and reasoning, and evaluates these three components across standards that represent the variation within a mid-level undergraduate cohort.

Using this rubric, we evaluated the ability of undergraduate science students to communicate scientific arguments in an oral presentation task in which they presented data acquired from an inquiry-based practical (Bugarcic, Zimbardi, Macaranas & Thorn, 2012). Students demonstrated the ability to make claims, supply evidence and articulate reasoning that linked claims with supporting evidence. However, the standard of these elements was varied, and the structure of students’ arguments was not always complete. Using an action-research approach, these initial findings were used to develop student guidelines and alter the curriculum in a subsequent iteration of the course. This intervention resulted in students presenting more complete and higher quality arguments. Overall, this study reports on the development of the rubric and describes the design and impact of an evidence-driven teaching intervention that enhances students’ scientific argument development in oral presentations.

SYM-34-04

DEVELOPING STUDENTS’ SCIENTIFIC WRITING SKILLS THROUGH EMBEDDED WRITING ACTIVITIES IN BIOSCIENCE UNDERGRADUATE LABORATORY AND HONOURS COURSES

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Scientific writing skills are important for a science career, yet specific training can be difficult to integrate effectively into a University program. To challenge science students to improve their writing we incorporated writing activities into undergraduate biological science laboratory courses and into Honours courses, and designed appropriate feedback mechanisms. The outcome is that students experience cycles of writing, receiving feedback and making improvements, which continue until both student and assessor are satisfied with the outcomes. This proactive approach encourages students to change their attitude towards scientific writing, which in turn promotes critical thinking and independent learning. Embedding the writing activities into existing courses enables the students to see the relevance of the writing training and students enthusiastically embraced the iterative feedback processes. We probed student opinions regarding writing and their perceived value of the writing exercises by anonymous pre- and post-course surveys using a combination of closed and open questions. The courses were also evaluated through the University’s standard course evaluation surveys.

This study describes the ability of the scientific argument and writing and performing simple writing tasks significantly improved after experiencing the writing activities. Independent assessors evaluated the standard of students’ written reports that originated from the same laboratory course held in years before and after writing activities were incorporated into the curriculum. The assessors reported a significant improvement in scientific writing quality that correlated with the increase in student confidence and attitudes towards writing.

SYM-34-05

BEYOND MEMORISING FACTS: DEVELOPING CRITICAL THINKING AND SCIENTIFIC REASONING SKILLS IN BIOLOGY UNDERGRADUATES

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In 2012 we introduced two new second-year biology courses aimed at developing a number of generic skills expected in a biologist including, but not limited to, critical evaluation of the scientific literature, science writing and communication, critical thinking, experimental design and statistical analysis. Whilst the courses we describe here were designed specifically to focus on developing these skills, the teaching approach and assessment pieces could be integrated into existing courses. The course Big Questions in Biology presented a syllabus with a focus on the nure of science, using case studies to introduce fundamental scientific concepts and the relationship between biology and society. Assessment pieces in this course include a focus on improving writing skills in different contexts. The second course, Experimental Design and Analysis, focuses on improving student skills in the essential aspects of research from experimental design through to application of statistical tests, evaluation and analysis of data and presentation of outcomes. We embedded these learning outcomes in a biology context, making the course interesting and relevant for biologists. Curriculum design of both courses and experiences of the ‘Second year biology’ student will be presented.

SYM-35-01

LIVE FAST, DIE YOUNG? PLANT TRAITS ASSOCIATED WITH RECOVERY FROM DROUGHT-INDUCED XYLEM EMBOLISM ACROSS CONTRASTING BIOMES

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Background/Aims Drought intensity may increase in future climates in many regions, causing xylem embolism, which may accumulate to lethal levels, if not refilled. Plant species that allow xylem embolism may avoid drought-induced mortality. Interestingly, many species exhibit significant night-time sap flux, which is hypothesized to contribute to refilling of xylem embolism. This study relates recovery from drought-induced xylem embolism to night-time water flux. Here, I droughted 80 woody plant species (spanning tropical to semi-arid biomes) and measured xylem vulnerability curves and subsequent recovery from embolism following re-watering. I calculated a xylem recovery index (XRI; percent loss of conductivity (PLC) during drought relative to PLC twelve hours following re-watering) to quantify hydraulic recovery following drought. I tested whether (1) xylem recovery was linked with delayed time to mortality, (2) xylem recovery was linked with nocturnal stomatal conductance, and (3) whether elevated CO2 increases time to death and Xylem Recovery Index. Whole-plant and leaf traits that influence xylem recovery, including stomatal conductance, transpiration, xylem embolism, turgor loss point, photosynthesis, both instantaneous (A/Gs) and integrated water use efficiency (CT3 estimated using carbon isotope), nonstructural carbohydrate content, parenchyma starch content, specific leaf area, and wood density are discussed. Results Counter-intuitively, species with the highest recovery from embolism also had fastest time to mortality. Also counter-intuitively, night-time stomatal conductance was positively related to xylem recovery. The strongest driver of xylem recovery was rate of xylem embolism, and plant species clustered by biome type. CO2 increased leaf temperature, branch diameter and branch conductivity and decreased stomatal conductance, leading to no overall impact on time to death or xylem recovery. Hydraulic strategies used by plants across various biomes to recover from xylem embolism, and how these vary across biomes to avoid drought-mortality are described. These results enable us to (1) predict which species will be resilient to drought-induced mortality, and (2) prioritise which whole-plant and leaf traits are most important to include in vegetation models.
SYM-35-02
COMPARATIVE ECOPHYSIOLOGY OF ACACIA HYBRID CLONES, DOES POLYPOLYOID INFERR INCREASED DROUGHT TOLERANCE?
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The evolutionary significance of polyploidy remains widely debated, however it is often assumed that polyploidy is associated with traits that increase the adaptive capacity of plants to environmental stresses such as water stress. Here we examined whether tetraploid plants have increased capacity to adapt to future climatic stresses than their diploid counterparts. An improved understanding of this could help infer inraspecific responses to increasing climatic variability. In this study we examined the comparative ecophysiology of diploid and tetraploid Acacia clones. In particular we examined the response of these clones to terminal drought through comparison of leaf gas exchange, leaf and whole plant hydraulic conductance and tissue carbohydrate concentrations. All clones experienced significant loss of hydraulic function and this was associated with depletion of carbohydrate stores across all tissues. Differences among clones were apparent and these provide insights to within species variability in these drought tolerance traits. While preliminary in nature these insights could assist in selection of genotypes best suited for novel or future environmental conditions.

SYM-35-03
TESTING THE LIMITS OF PHOTOSYNTHETIC PLASTICITY IN TROPICAL AND TEMPERATE PROVENANCES OF TWO WIDESPREAD EUCALYPTUS SPECIES
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Eucalypts dominate Australia’s native forests where almost 800 species occupy approximately 75% (92 million hectares) of total native forest area and extend over broad bioclimatic gradients. Future climate warming events as a consequence of global climate change will increase the mismatch between trees and their habitat, potentially leading to forest decline. As sessile organisms, the ability of trees to cope with climate change depends largely upon their physiological and biochemical plasticity. We undertook a series of glasshouse studies aimed at elucidating the mechanisms of thermal photosynthetic acclimation in eucalypts and to test whether warm-origin provenances are less plastic and more sensitive to warming than cool-origin provenances. For this study, four provenances of E. tereticornis and three of E. grandis contrasting temperatures environments along the Eastern Australian coast were grown at temperatures equivalent to their mean annual “home” and warmed (home + 3.5°C) temperature. We tested the influence of “home” climate on the adaptive ability of the tree seedlings to thermally acclimate. We measured the response of leaf photosynthesis to CO2 (A–Ci curve) at 4–5 temperatures in order to derive estimates of Vcmax, Jmax and their respective thermal dependence activation energies. Metabolic Michaelis-Menten kinetics constants (eCO2) and Kv were measured for comparisons with the in vivo determined parameters. Leaf contents of nitrogen, proteins and Rubisco were determined on gas exchange leaves. Our data showed that E. tereticornis had higher light saturated photosynthetic rates (Amax) and capacity (Vcmax, Jmax) than E. grandis. Growth under warming (3.5°C) showed little impact on photosynthesis of provenances of either species. Calculations of temperature optimum of photosynthesis (Tcmax) determined from Amax was unchanged at ~32°C for E. grandis, however for E. tereticornis, Tcmax was ~28°C within cool provenances and 32°C in warm provenances. Overwintering and gas exchange parameters at an ambient temperature of 15±2°C showed photosynthesis was lower in warmer-origin relative to cool-origin provenances and this was reflected in lower Rubisco Km across warmer origins compared to cool origin counterparts. Therefore, photosynthesis in Eucalypt provenances acclimated similarly to warming indicating that Eucalypts are likely to maintain positive carbon gain in warmer climates if resources such as water and nutrients are available.

SYM-35-04
SHORT-TERM AND LONG-TERM EFFECTS OF CO2 ON MESOPHYLL CONDUCTANCE IN WHEAT (TRITICUM AESTIVUM L.)
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The rising atmospheric CO2 concentration is altering global temperature and precipitation patterns, which could challenge agricultural productivity. Mesophyll conductance (gM) is now recognized as an important limiting process for photosynthesis and has been found to respond to both environmental conditions and leaf anatomical traits. To understand long-term (growth) and short-term (measurement) CO2 effects on gM, two cultivars (Crannbrook and Halberd) of wheat were grown at four different CO2 concentrations (206, 344, 489 and 1085 μmol mol−1) in controlled-environment cabinets. A mild water limitation was imposed to assess the interactive effects of growth CO2 and drought for the two genotypes. gM responded to both environmental conditions and leaf anatomical traits. While preliminary in nature these insights could assist in selection of genotypes best suited for novel or future environmental conditions.

SYM-35-05
ASSESSING THE COORDINATION OF LEAF HYDRAULIC SUPPLY AND DEMAND WITH GAS EXCHANGE IN RESPONSE TO ELEVATED CO2 ACROSS GENOTYPES OF EUCALYPTS CAMALDULENSIS SEEDLINGS
Blackman C.J.1, Smith R.1, Pinkard L.2, O’Grady T.2, Farquhar G.3 and Tissue D.T.1
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The functional links between the hydraulic traits of leaves and maximum rates of transpiration and photosynthetic gas exchange are widely recognised across plant groups. However, the strength of these relationships has rarely been tested across genotypes within a single species. Furthermore, little is known if leaf hydraulics and gas exchange parameters will show a coordinated response to climate change, including rising atmospheric CO2. In this study, we examined the coordination of key structural and functional traits related to leaf water supply (vein density), water demand (stomatal density) and gas exchange parameters among 10 genotypes of Eucalyptus camaldulensis across 4-5 temperatures in order to derive estimates of Vcmax, Jmax, Ks, Kv and Km that increase the adaptive capacity of plants to environmental stresses

However, our findings also suggest that increased vein density in response to elevated CO2 may contribute to an increase in photosynthetic rate and overall plant productivity.
SYM-36-01
FROM BIOCHEMISTRY TO BIOMATERIALS: BUILDING AND REPAIRING TISSUE IN 3D
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We are utilizing decoded biological rules of assembly to fabricate detailed, 2D and 3D elastin structures. Its translation is yielding increasingly sophisticated elastic biomaterials, tailored to match the elastin-rich tissues they seek to replace. Massive molecular protein assemblies are organized facilitators of endothelial progenitor and subsequent endothelial attachment, spreading and proliferation, accompanied by modulated smooth muscle cell colonization without restenosis. Additionally, we have adapted the structural asymmetry and integrin binding polarity of the elastin monomer, tropoelastin, to decorate polymers for dermal and vascular applications to confer beneficial cell interactive and low thrombogenic properties. We covalently transferred these features to a panel of polymers. Combining tropoelastin with both synthetic and natural polymers further expands the potential uses of this versatile biomolecule. We find that this combined treatment enhances human dermal fibroblast and human endothelial cell attachment, cytoskeletal assembly and viability, combined with elevated cell junction PECAM-1 staining. Tropoelastin represents a unique class of molecule that achieves the desired physiological response of increasing endothelialisation while conferring low thrombogenicity, so is exquisitely suited as a coating for flexible polymers and construction material for vascular conduits.

SYM-36-02
HARNESSING THE REGENERATIVE POWER OF THE BRAIN USING BIOMATERIALS
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Currently there is no effective therapy that can assist repair of the damaged brain. Current treatment strategies of damaged brain tissue resulting from trauma, stroke, aging and neurodegenerative diseases are based on principles of enhancing survival and function of the remaining intact tissue. Furthermore, intrinsic self-repair mechanisms are very limited in the adult brain, and highly variable depending on the size, location and mode of injury. Regrowth of neurons, particularly over large distances is actively suppressed in the adult brain. Despite the lack of tissue repair following brain injury, partial recovery of neurological function is still possible due to the intrinsic neural plasticity that is retained in the adult brain, which enables structural remodelling at the level of local synapses, and a degree of functional recovery. This presentation will initially focus on the development of nanofibrous scaffolds and hydrogels that have been engineered to be injected into the brain for the repair of neural pathways. Nanofibres were produced using a process called electrospinning, and our research has determined critical architectural and biofunctional properties of nanofibres that control both the migration of cells as well as the extent of inflammation and integration into the brain. Concurrently we developed hydrogels based on ECM proteins and peptides that slowly release neurotrophins to enhance the regenerative ability of the brain. These scaffolds were implanted as a tract in the brain to deliberately disrupt the subventricular zone, a highly localized region containing neural stem cells. The resident neural stem cells could be diverted away from their natural pathways in a controlled and concerted manner. Our research further demonstrated that neural stem cells were able to infiltrate the nanofibrous scaffold and be directed along the implant tract to distant brain regions where they gave rise to new neurons.

SYM-36-03
OPTIMISATION OF STEM CELL EXPANSION, MAINTENANCE AND DIFFERENTIATION USING MULTIPLEXED MICROBIOREACTOR ARRAYS
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Effective use of human stem cells in regenerative medicine and drug screening depends on our ability to effectively direct both their undifferentiated expansion and differentiation into desired lineages. Exquisite control over stem cell fate is needed to efficiently produce sufficient, defined cell populations for such applications, yet this is substantially hindered by undefined culture components, signal crosstalk between multiple exogenous and endogenous factors, and spatiotemporal variations in microenvironmental composition inherent to conventional culture formats. We have recently developed scalable, vessel-based, multi-chamber perfusion technology, the multiplexed microbiorreactor arrays (MBA) that provide a full-factorial set of exogenous factor compositions, and also allow controlled accumulation of paracrine factors. These MBAs have been used to survey up to 8100 individual, perfused cellular microenvironments in parallel. Through screens of pluripotency maintenance and mesendodermal differentiation of human pluripotent stem cells (hPSCs) as examples, we demonstrate the unique ability of this platform to separate, visualise, identify and modulate paracrine effects that are not otherwise readily accessible. In addition, we have applied this same MBA platform to the optimisation of small molecule agonists and antagonists on mesenchymal stem cell osteogenic differentiation outcomes. With our 8100-chamber, high density microbiorreactor array, we have assessed the impacts and interplay of small molecule agonists and growth factors on human cardiomyocyte proliferation. Culture conditions optimized with the MBAs have been shown to be readily translatable to improving maintenance or differentiation in conventional culture protocols, exemplifying the immediate practicality of the MBA. These multiplexed MBAs decipher factor interplay and signalling hierarchies that control stem cell fate, and are applicable as a universal microenvironmental screening platform for bioprocess optimisation, media formulation design, quality control for cellular therapeutics and cell-based drug toxicity and stratification.

SYM-36-04
BIOMIMETIC MACROPOROUS HYDROGELS FOR CELL CULTURE AND SOFT TISSUE ENGINEERING
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Biomaterial constructs have been proposed to fulfil many important roles in tissue engineering, including maintaining space for tissue growth, acting as scaffolding for cell attachment and migration, mimicking native tissue microenvironments and delivering bioactive molecules. However, they are not always successful in vivo and can even impede the desired tissue development. Optimal design of biomaterial constructs depends on insights into the complex cell and tissue requirements during tissue regeneration, their interactions with biomaterials and how these change over time during development of tissues. We use characterisation of the mechanical properties of cells and tissues as a basis for the design of biomaterial constructs for stem cell culture in vitro and tissue engineering of soft vascularised tissues in vivo. Hydrogels, hydrophilic polymer networks that have high water contents, can be fabricated with similar viscoelastic properties to those of native tissues. Highly interconnected macroporosity is introduced into such hydrogels to facilitate molecular and cellular transport as well as vascularisation for tissue engineering. Macroporous hydrogels have been fabricated from natural biopolymers including hyaluronic acid and chitosan using templating, gas foaming and cryogelation strategies to control their elasticities, pore sizes and interconnectivity to suit a range of soft tissues. By combining physical, chemical and biological factors in the design of biomaterial constructs, we aim to enhance the growth rate and quality of tissue engineering outcomes.

ComBio2014 • Canberra, ACT, Australia • 28 September – 2 October, 2014 • PAGE 69
SYM-36-05
USING PANCREATIC CELL BIOLOGY TO INFORM THE DESIGN OF A BIOSYNTHETIC HYDROGEL MICROENCAPSULATION SYSTEM
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The ultimate objective of any cell encapsulation system is the reversal of the clinical condition being treated. Type 1 diabetes is an ideal application for this treatment. While islet transplantation alone has the potential to cure type 1 diabetes, long term outcomes remain poor. One reason for this lack of success is that when islets are isolated they are removed from native extracellular matrix (ECM) which ultimately results in cell death due to absence of cell-matrix interactions. Therefore, one of the most important requirements in designing a microencapsulation system is the ability to keep the encapsulated cells alive and functioning. To address this requirement, we aimed to characterise the native islet ECM, to identify critical components for islet cell support and to develop a biosynthetic hydrogel microencapsulation system. Healthy pancreatic tissue from several species was used, and numerous glycosaminoglycans (GAG) and proteins were investigated. Only heparan sulfate (HS) was immunolocalised on the cell surface of insulin-positive β cells, and not in the surrounding acinar cells. HS has also been proposed by others as critical for survival of β cells. Therefore, heparin (as a model of HS) was copolymerised with poly(vinyl alcohol) (PVA) to form biosynthetic gels. MIN6 murine insulinoma cells (107 cells/ml) were mixed with the polymer solutions before they were polymerised, and smooth spherical microspheres were generated. The encapsulated cells remained viable for up to 28 days. Metabolic activity measured via ATP production decreased to non detectable levels by day 14 in spheres with PVA alone, but cells were still active at day 28 in PVA/heparin spheres. This study demonstrated that HS inclusion in biosynthetic hydrogel microspheres is a promising approach for supporting islet cell recovery following isolation and for improved transplant survival.

SYM-37-01
A BUBBLE RIDE FOR THE ONCOGENES: EXOSOMES IN THE TUMOR MICROENVIRONMENT
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Extracellular vesicles are signaling organelles that are released by many cell types and are highly conserved in both prokaryotes and eukaryotes. Based on the mechanism of biogenesis, these membranous vesicles can be classified as exosomes, shedding microvesicles and apoptotic blebs. It is becoming clearer that these extracellular vesicles mediate signal transduction in both autocrine and paracrine fashion by the transfer of proteins and RNA. Whilst the role of extracellular vesicles including exosomes in pathogenesis is well established, very little is known about their function in normal physiological conditions. Recent evidences allude that extracellular vesicles can mediate both protective and pathogenic effect depending on the precise state. For tumors to progress, bidirectional crosstalk between tumor cells and the tumor and its surrounding supporting tissue. Recent literature indicated that all aspects of cellular tumorigenicity are profoundly influenced by reciprocal interactions between responding normal cells, their mediators, structural components of the extracellular matrix, and genetically altered neoplastic cells. Extracellular vesicles have been recently recognized as important mediators of the cross-talk in the tumor microenvironment with both pro- and anti-tumorigenic properties.

SYM-37-02
COMPREHENSIVE LIPIDOME PROFILING TO IDENTIFY FUNCTIONAL BIOMARKERS OF COLORECTAL CANCER MALIGNANCY AND METASTATIC PROGRESSION
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The onset and progression of various diseases, including cancer, are known to be associated with the disruption of lipid metabolism or signaling pathways. To date, however, systematic identification and quantification of the diverse range of individual molecular lipid species that may be present within cancerous cells or tissues, and exploration of their functional roles in malignancy and metastatic progression, has not been broadly explored. Here, a comprehensive lipidome analysis strategy, consisting of a monophase extraction technique and novel in-situ sequential functional group specific chemical derivatization reactions, coupled with high-resolution ‘shoutgun’ mass spectrometry and ‘targeted’ tandem mass spectrometry (MS/MS), has been developed and applied to the comprehensive identification, characterization and quantitative analysis of ~1000 individual lipids across a broad range of lipid classes and sub-classes (glycerolipids, glycerophospholipids, sphingolipids and sterol lipids), and across more than 4 orders of magnitude dynamic range of abundance, between the primary colon adenocarcinoma cell line, SW480, and its isogenic metastatic derivative, SW620. Significant changes in abundance and/or lipid subclass remodelling of numerous lipid classes were observed, including for monoalkyl-glycerophosphocholine (PC) and monoalkenyl-glycerophosphoethanolamine (PE) ether lipids, and certain long chain length SM species, that have previously been associated with cancer development, or are known mediators in a range of physiological and pathological processes. Based on these results, siRNA knockdown of the rate limiting peroxisomal enzyme for ether lipid biosynthesis, alkylglyceronephosphate synthase (AGPS), followed by comprehensive lipidome profiling and PCR array analysis, is demonstrated for the first time to significantly attenuate multiple genes known to be associated with molecular and phenotypic characteristics of cancer malignancy and metastasis.

SYM-37-03
QUANTITATIVE PHOSPHOTYROSINE PROFILING TO IDENTIFY NOVEL THERAPEUTIC TARGETS IN HIGH-RISK PEDIATRIC LEUKEMIA
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High risk pediatric acute lymphoblastic leukemia (ALL) is a sub group of ALL with very poor outcome and often associated with dysregulated cellular signaling mechanism. Genetic studies have also reported mutations and altered expression in the tyrosine kinase group of signaling molecules in high risk ALL patients. We have recently developed and established a stable isotope labelling of amino acid (SILAC) based quantitative phosphotyrosine profiling method for patient derived xenograft (PDX) model of paediatric ALL and profiled a panel of 16 high-risk ALL (PDXs). Using a mass spectrometry based approach we have identified and quantified close to 1500 tyrosine phosphorylated sites across 16 PDXs. Such profiling could accurately classify these leukemias into either T or B-cell lineages with the high-risk early T-cell precursor and Ph-like ALL clustering as a distinct group. We targeted the perturbed expression of ABL1, FLT3 and JAK in four xenografts using commercially available inhibitors and assessed the preclinical efficacy of the specific inhibitors in the PDXs. By quantitatively assessing the tyrosine phosphorylation state of activated signaling molecules in high risk ALL patients, we were able to identify and validate clinically relevant targets. This study highlights the application and potential of phosphotyrosine profiling for improved treatment strategies in high-risk pediatric ALL.
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SYM-37-04
MULTIPHASE ON-CHIP PEPTIDE SEPARATION ENABLES IMPROVED IDENTIFICATION AND QUANTITATION IN TARGETED AND DISCOVERY PROTEOMICs

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Chemical or physical fractionation of biological samples is an essential step to enable proteome characterization using mass spectrometry. Most fractionation strategies are time consuming and involve multiple manual sample handling steps and are thus prone to significant losses. Here we report development of an online, chip-based multi-phase peptide fraction workflow based on ion exchange and reversed-phase separations. In this hands-free operation the autosampler is used to deliver salt plugs of increasing concentration to elute tryptic peptides from the ion exchange media onto an analytical reversed-phase column that is in-line with the mass spectrometer. This sample fractionation protocol provides significant improvements in peptide detection and quantitation compared to conventional analyses.

We demonstrated the utility of the method with two examples. First, we coupled the multiphasic chip with the SWATH-MS data independent workflow to generate a spectral library for phenotyping melanoma cells. Using only 5ug of sample we produced a spectral library containing over 15500 unique peptides, 25% more peptides and one-fifth less sample consumed, than that achieved using equivalent MS sequencing time with conventional 1D LC-MS/MS. Using the spectral library and SWATH-MS workflow we were readily able to identify those cell lines that showed resistance to MEK inhibitors, independent of genotype. A second demonstration of the multiphasic chip was shown with targeted quantitation using selected reaction monitoring. By conducting on-chip fractionation we demonstrated improved peak height and peak area for more than 50% of targeted peptides compared with conventional LC-SRM. This represents a useful approach for biomarker detection to improve sensitivity in complex matrices. In summary, on-chip, multiphase peptide fractionation is a fully automated approach that improves comprehensive proteome analysis.

SYM-38-01
CAGING THE BEAST: HOW FBP, FIR AND DNA SUPERCOILING CONTRAIN MYC EXPRESSION AND FUNCTION

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The nuclear proto-oncogene MYC is deregulated in most cancers. MYC binding and action across the genome is only weakly predicted by the distribution of its high affinity binding site, the E-box. Rather MYC associates with the promoters of already active genes according to their level of expression to amplify ongoing transcription. Thus MYC supports cellular state, but can drive transitions by driving pathways across critical thresholds. Because of this amplifier activity, MYC expression must be tightly controlled. There is no final common input to the MYC promoter; rather a multitude of pathways operating through diverse sets of transcription factors in different cells drives the promoter.

Once the MYC promoter is activated, its output is modulated by the Far Upstream Element Binding Protein (FBP) that sequence selectively engages FUSE, a single-stranded element of the MYC promoter. FUSE melts in response to dynamic supercoiling torque generated by ongoing transcription and melted-FUSE recruits both FBP and the FBP Interacting Repressor (FIR) to adjust MYC-expression up or down in real-time. Thus FBP and FIR help to mechanically integrate the diverse inputs onto the MYC promoter. Upon knockout of FBP in mice, MYC levels fluctuate from embryo to embryo. Methods that we developed to monitor the disposition of supercoiling, alternative DNA structures, and topoisomerase binding and activity across the genome indicate that the same principles governing the dynamic interplay between DNA topology and structure with transcription and chromatin at the MYC locus operate throughout the genome. Understanding the DNA mechanics of the genome as well as its physical and systems organization promises to reveal new ways to modify gene expression in health and disease.

SYM-38-02
TRANSCRIPTION FACTORS: CHOOSING THE RIGHT TARGET GENE

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Transcription factors are typically composed of a DNA-binding domain and a functional (activation or repression) domain. The DNA-binding domain is such that it is sequence-specific and is able to deliver the transcription factor to target genes containing its cognate recognition site. Nevertheless, there is substantial evidence that the sequence-specificity of DNA-binding domains alone cannot explain the genomic profile of protein–gene interactions observed in vivo. For instance, different transcription factors of the same family, sharing near identical DNA-binding domains, bind different target genes in vivo. Additionally, functional domains are known to make protein–protein interactions which may be important for targeting the transcription factor to certain gene regulatory regions. Finally, it has been observed that in vivo transcription factors only recognize a fraction of existing DNA-consensus sites, again suggesting that other processes must be in operation. We have been directly investigating the role of the functional domain of KLF3. KLF3 is a typical zinc finger transcription factor with a clearly identifiable zinc finger and functional domain. We have carried out ChipSeq experiments on a series of KLF3 mutants. The data showed that the functional domain of KLF3 plays a major role in facilitating the delivery of KLF3 to target genes in vivo. We have also grafted this KLF3 domain onto a completely artificial zinc finger protein and again found that the functional domain of KLF3 affects targeting specificity in vivo. In summary, we conclude that the functional domains of naturally occurring zinc finger proteins are likely to play an important role in determining the genomic profile of transcription factors.
Epithelial-mesenchymal transitions (EMT) and mesenchymal-epithelial transitions (MET) are essential for embryonic development and for adult organisms to respond to tissue injury. They have also emerged as key processes during cancer development and progression, associated with increased tumor cell plasticity. EMT/MET events involve extensive transcriptional reprogramming, which our recent work indicates is critically dependent on Activator Protein-1 (AP-1) transcription factor complexes. In particular, our data highlight a central role for FRA1, an AP-1 family member whose induction by various oncogenes promotes highly malignant phenotypes. We found that FRA1 activity is required both for establishing and maintaining EMT in tumor cell lines, which is coupled to its direct regulation of gene networks orchestrating EMT/MET equilibriums, such as the TGFβ/ZEB axis. By characterizing the composition of FRA1/AP-1 complexes in tumor cells, we are now also beginning to identify novel regulators of the pro-mesenchymal actions of FRA1/AP-1, including Smad proteins and protein phosphatase 2A.

**SYM-38-05**

**CROSSTALK BETWEEN SINGLE-MINDED 2 AND HYPOXIA INDUCIBLE FACTORS IN CANCER DEVELOPMENT**

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Single-minded 2 (SIM2) is a basic Helix-Loop-Helix (bHLH) Per-ARNT-SIM (PAS) transcription factor which forms a functionally active heterodimer when dimerised with Aryl hydrocarbon Receptor Nucleolar Translocator (ARNT). SIM2 is frequently upregulated in prostate cancers and correlates with increased tumour aggressiveness and decreased patient survival rate. Conversely, decreased expression of SIM2 during breast cancer development is associated with cellular de-differentiation and promotion of metastasis. The context-specific function of SIM2 in these cancers is still poorly understood, including both direct targets of SIM2 transcriptional regulation and the effects of aberrant SIM2 expression on the function of other bHLH PAS factors. The bHLH PAS transcription factor Hypoxia Inducible Factor 1α (HIF1α) is critical to cancer survival and development within the hypoxic tumor environment. SIM2 and HIF1α compete for binding to ARNT (also known as HIF1β), and SIM2-ARNT heterodimers have also been observed to bind hypoxic response elements (HREs) to repress expression of reporter genes and the endogenous HIF1 target BNIP3. In this study we use a novel lentiviral expression vector based on the Tet-On 3G system to inducibly and reversibly express ectopic SIM2 in several prostate and breast cancer cell lines, and perform microarray analysis to assess transcriptome changes with altered SIM2 expression in different oxygen conditions. ChIP studies and genome editing techniques further characterise novel, tissue-specific targets of SIM2 activity in cancer, regulated both directly and as a result of crosstalk with HIF.

**SYM-38-03**

**ACTIVATOR PROTEIN-1: A HUB FOR EPITHELIAL-MESENCHYMAL PLASTICITY CONTROL IN CANCER CELLS**

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Epithelial-mesenchymal transitions (EMT) and mesenchymal-epithelial transitions (MET) are essential for embryonic development and for adult organisms to respond to tissue injury. They have also emerged as key processes during cancer development and progression, associated with increased tumor cell plasticity. EMT/MET events involve extensive transcriptional reprogramming, which our recent work indicates is critically dependent on Activator Protein-1 (AP-1) transcription factor complexes. In particular, our data highlight a central role for FRA1, an AP-1 family member whose induction by various oncogenes promotes highly malignant phenotypes. We found that FRA1 activity is required both for establishing and maintaining EMT in tumor cell lines, which is coupled to its direct regulation of gene networks orchestrating EMT/MET equilibriums, such as the TGFβ/ZEB axis. By characterizing the composition of FRA1/AP-1 complexes in tumor cells, we are now also beginning to identify novel regulators of the pro-mesenchymal actions of FRA1/AP-1, including Smad proteins and protein phosphatase 2A.

**SYM-38-04**

**HISTONE VARIANT H3.3 PROVIDES THE HETEROCHROMATIC H3K9 TRIMETHYLATION MARK AT TELOMERES**

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The telomere DNA is enriched for typical heterochromatin marks such as H3K9me3 (H3 trimethylated lysine 9) and H4K20me3 (H4 trimethylated lysine 20). The heterochromatic nature of telomere chromatin is important for its structure and function. Our previous studies show that Alpha Thalassemia Mental Retardation X-linked (ATRX) and H3.3 are also key regulators of the telomeric chromatin. Deposition of H3.3 by ATRX and its interacting partner DAXX is essential for chromatin repression at the telomere. Function of ATRX-H3.3 in maintaining telomere chromatin repression is further demonstrated by recent studies that show a strong association of ATRX mutation with alternative lengthening of telomeres (ALT) in human cancers. Here, we used h3f3a and h3f3b knockout cell models to investigate the role of H3.3 function in controlling repressive chromatin assembly at the telomeres. We show evidence that K9 residue of H3.3 is methylated by SUVAR39H1/2 and H3.3K9me3 to provide as a repressive histone mark at telomeres. In H3.3 knockout cell lines, a deficiency in H3.3 results in reduced levels of heterochromatic marks including H3K9me3, H4K20me3 and ATRX at telomeres. Furthermore, these cells showed a greater increase in telomere DNA damage and telomere sister chromatid exchange (t-SCE), following treatment with replication drug, suggesting their sensitivity toward replication stalling. Together, our study shows for the first time that H3.3 is utilized as a heterochromatin histone mark, via trimethylation of its K9 residue. And, we show that a sufficient H3.3 supply is essential for the maintenance of telomere chromatin integrity, in particular, in the presence of replication difficulty. Our study provides not only insights into the role of H3.3 in the control of epigenetic inheritance at heterochromatin domain, but also a clear evidence that Histone H3.3 can act as a repressive chromatin mark, instead of a histone mark associated only with actively transcribed chromatin.

**SYM-39-01**

**THE STRUCTURAL BIOLOGY OF THE INSULIN / INSULIN RECEPTOR INTERACTION - NEW INSIGHTS AT THE LIMITS OF RESOLUTION**

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The structural biology of the insulin-insulin receptor interaction has until very recently [1] proved to be elusive. Here, we describe the changes that occur in both the hormone and the receptor upon insulin engaging its primary binding site on the receptor surface. In particular, we show that the hormone’s B-chain C-terminal strand undergoes a hinge-like conformational change upon receptor engagement. In addition, we present data that suggest that this conformational change is integrally involved in protecting the hormone from inappropriate aggregation during its conformational life-cycle. [1] Menting et al. (2013). Nature, 493, 241-5.
SYM-39-02
MECHANISM OF ACTIVATION OF JAK2 BY THE GROWTH HORMONE RECEPTOR

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While human GH was first isolated seventy years ago, it is only now that we have reached a clear understanding of the molecular basis for its action. Along the way, GH has been shown to act on virtually all cells in the body, and to have many important actions apart from promoting somatic growth. These range from its textbook role in opposing insulin action in glucose utilization through prevention of hepatic steatosis and promotion of helper T4 cells number in AIDS, to stimulation of neural stem cell replication in response to exercise, thus enhancing cognition. Identifying loss of function mutations in Laron dwarfism has also allowed us to conclude that loss of GH receptor confers resistance to death from cancer in humans and rodents. The GH receptor was the first single transmembrane pass receptors which signal as dimers through associated Janus (JAK) tyrosine kinases to the Signal Transducers and Activators of Transcription (STATs). Here we will describe how we have elucidated the molecular movements of the GH receptor consequent to GH binding to its receptor, and how these movements lead to activation of JAK2, which then activates STAT5 by phosphorylation, resulting in transactivation of key metabolic and growth related genes. We also present structural data on the cytoplasmic domain of the receptor relevant to its signalling actions. Finally, we have shown that a second tyrosine kinase, a src family kinase, is recruited independently of JAK2 by the GH receptor, and this has key consequences for immunotolerance and liver regeneration. Supported by NHMRC.

SYM-39-03
EXPLOITING GP130 SIGNALING FOR THE TREATMENT OF GASTROINTESTINAL CANCER

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Among the cytokines linked to inflammation-associated cancer, interleukin (IL)-6 drives many of the ‘cancer hallmarks’ through downstream activation of the gp130-STAT3 signaling pathway. STAT3 activation is linked to poor survival in patients with cancer and is thought to arise primarily from elevated IL-6 [1]. Accordingly, inhibitors of IL-6 signaling are in clinical trials for a number of epithelial cancers. However, we show that the related cytokine IL-11 has a stronger correlation with elevated STAT3 activation in human gastrointestinal cancers. Using genetic mouse models, we reveal that IL-11 has a more prominent role compared to IL-6 during the progression of sporadic and inflammation-associated colon and gastric cancers [2]. Accordingly, in these models and in human tumor cell line xenograft models, pharmacologic inhibition of IL-11 signaling alleviated STAT3 activation, suppressed tumor cell proliferation, and inhibited the invasive capacity of neoplastic cells and reduced growth of human tumor cell line xenografts. Importantly, IL-11 inhibition had no impact on hematopoiesis, an undesirable side effect of systemic STAT3 inhibition. Our data provide support for the clinical development of IL-11 signaling antagonists for the treatment of gastrointestinal cancers. [1] Ernst et al., Seminars in Immunology 2014 [2] Putoczki et al., Cancer Cell 2013.

SYM-39-04
A UNIQUE INSIGHT INTO THE MECHANISM OF INSULIN RECEPTOR BINDING AND ACTIVATION USING DICARBA INSULIN PEPTIDES

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Treatment of diabetes depends on the use of insulin compounds that effectively lower blood glucose through activation of insulin receptor signaling. We designed a series of short- and long-acting insulin analogues with an A6-A11 dicarba bond with the intention to create more stable analogues. Concern was raised recently over some insulin analogues that have greater potential than insulin to activate mitogenic signaling. Remarkably, through synthesis of dicarba insulins we have generated two isomers that represent the active and inactive conformations. Through a series of receptor binding/activation assays, biological assays and biophysical analyses we have characterised these two isomers. Interestingly, by simply substituting the disulphide bond at A6-A11 to a dicarba bond, some of these molecules become significantly less potent in promoting mitogenic activity signaling through IGF-1R and IR (DNA synthesis assay), while maintaining the metabolic activity via IR (in vitro glucose uptake assays and in vivo glucose tolerance tests). Spectral analyses suggest that the active isomer has a unique conformation and that we have a completely new insight into the way in which insulin binds its receptor.

SYM-39-05
THE PHOSPHOLIPID FLIPASSE DATP8B IS REQUIRED FOR ODORANT RECEPTOR FUNCTION IN DROSOPHILA

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The olfactory systems of insects are fundamental to all aspects of their behaviour, and insect olfactory receptor neurons (ORNs) exhibit exquisite specificity and sensitivity to a wide range of environmental cues. In Drosophila ORN responses are determined by three different receptor families, the odorant receptor (Or), ionotropic-like (IR) and gustatory (Gr) receptors. However, the precise mechanisms of signalling by these different receptor families are not yet understood. We have found that the type 4 P-type ATPase phospholipid transporter dATP8B, the homologue of a protein associated with intrahepatic cholestasis and hearing loss in humans, is crucial for Or signalling. Our findings thus reveal a novel and unexpected requirement for lipid bilayer asymmetry in the signalling of a specific family of chemoreceptor proteins, and highlight the differences between the signalling mechanisms of the three insect olfactory receptor families. To further explore the mechanism by which dATP8B controls Or signalling we are biochemically investigating its lipid substrate specificity by expressing it in the yeast Saccharomyces cerevisiae. We are also exploring if dATP8B has other roles, and extending this work to determine the extent of functional redundancy in the family of six phospholipid flippases in Drosophila.
SYM-40-01

MOLECULAR RESPONSES OF MAIZE PLANTS TO NITROGEN OR PHOSPHATE STARVATION

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Yield of crop plants is strongly dependent on nitrogen availability in the soil and nitrogen use efficiency of the plant. However, knowledge about regulation of adaptive responses to nitrogen deprivation still mainly derives from the study of model species. In this study, the metabolic adaptation of maize source leaves to different nitrogen regimes was analyzed by parallel measurements of transcriptome and metabolome data. Initially, inbred lines A188 and B73 were cultivated under sufficient (15mm) or limiting (0.15mm) supply with nitrogen for up to 30 days. Nitrogen starvation caused the selective down-regulation of processes involved in nitrate reduction and amino acid assimilation; ammonium assimilation related transcripts on the other hand were not affected. Carbon assimilation related transcripts were characterized by high transcriptional coordination and general down-regulation under low nitrogen conditions. Nitrogen deprivation caused a slight accumulation of starch, but also directed increased amounts of carbohydrates into the cell wall and secondary metabolites. Under nitrogen deficiency, leaves accumulated large amounts of phosphate. Transcriptome data revealed that this was accompanied by massive down-regulation of genes usually involved in phosphate starvation response, underlining the great importance of phosphate homeostasis control under stress conditions. In a second experiment, sixteen different inbred lines were subjected to the same nitrogen deficiency treatment and tested for differences in the responses of their metabolite profiles. The lines showed similar qualitative responses to the stress, but displayed quantitative differences. The relevance of these quantitative differences for the growth response under nitrogen deprivation is discussed.

SYM-40-02

ALLEVIATING MICRONUTRIENT DEFICIENCIES THROUGH BIOFORTIFIED BANANAS

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Unacceptably high levels of micronutrient deficiencies persist in many developing countries despite implementation of strategies such as food fortification and supplements. Increasing the levels of specific micronutrients such as pro-vitamin A and iron in staple foods, biofortification, is a very promising strategy to address these high levels. Bananas are staple foods particularly in Africa and, in Uganda for example, the average consumption of bananas is 0.5kg per person per day. Vitamin A deficiency is high in Uganda and the cooking bananas are low in pro-vitamin A (PVA). We have genetically modified bananas to elevate the levels of pro-vitamin A produced in the fruit with a target of 20μg/g β-carotene equivalents dry weight. These plants were assessed in two field trials, in north Queensland. The “Golden Rice” strategy resulted in elevated PVA but often with undesirable phenotypic effects. However, there are bananas with naturally very high PVA. We utilised a number of genes from a PNG banana Asupina. The highest PVA level in a field grown transgenic line was a bunch with 73.8μg/g β-carotene equivalents dry weight compared with non-transgenic controls averaging 5.1μg/g. The highest line averaged 55.0μg/g. This line was transformed with a single PVA transgene, phytoene synthase, isolated from a banana with naturally high PVA. In contrast, there are no reported high iron bananas. We have tested a range of transgenes for iron enhancement. The most promising of these is the rice nicotianamine synthase gene used to develop high iron rice. The next generation of iron biofortification constructs are now in field trial in Australia and the PVA biofortification constructs have been transformed into local cultivars in Uganda and are now in field trial. The highest PVA line generated in Uganda had a fruit PVA content of 35μg/g.

SYM-40-03

OILSEED TAG YIELDS FROM PLANT LEAVES

CSIRO Agriculture Flagship, Canberra, ACT, Australia.

Demand for plant oils is projected to increase rapidly as the population grows in the coming decades. Limitations on arable land and other inputs mean it may be difficult to meet this additional demand with current oilseed-based production systems. In response, there has been significant research investment in the production of high biomass plants with elevated triacylglycerol (TAG) content for both food and oleochemical (fuel and feedstock) applications. In this presentation we will describe the metabolic engineering of Nicotiana spp. events which has resulted in the accumulation of over 30% storage oil (triaclyglycerol) in leaf tissue on a dry weight basis. This remarkable feat was achieved by combining multiple oil increase technologies in a coordinated and integrated metabolic engineering approach to overcome general ‘Push’ (fatty acid synthesis), ‘Pull’ (TAG assembly) and ‘Protect’ (oil storage) limitations in plant cells. This talk will also discuss recent successes in modifying the fatty acid profile of high-oil transgenic leaf tissue including the accumulation of high value unusual fatty acids as renewable chemical feedstocks.

SYM-40-04

A VACUOLAR INVERTASE, GHVIN1, CONTROLS COTTON FIBRE INITIATION PROBABLY BY MODULATING A SET OF REGULATORY GENES VIA SUGAR SIGNALLING

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Cotton fibres, the most important source of cellulose for the global textile industry, are single-celled trichomes initiated from the ovule epidermis at, or just prior to, anthesis. Fibre initiation establishes the number of fibres present on each seed; while the following elongation determines fibre length. Thus, the two processes are key determinants of cotton fibre yield and quality. We recently showed that vacuolar invertase (VIN) is an upstream regulator essential for fibre initiation (Wang et al., 2014 Plant J. 78:686-96), in addition to its role in fibre elongation (Wang et al., 2010 Plant Physiol. 154:744-56). The mRNA level of GHVIN1, a major VIN gene in cotton fibre, matched closely with VIN activity and fibre elongation rate. RNAi-mediated suppression of GHVIN1 resulted in a significant reduction of VIN activity in the seed epidermis, and consequently a fibreless phenotype, in a dosage-dependent manner. The absence of a negative effect on seed development indicates the phenotype is unlikely due to a lack of carbohydrate supply. Further analyses revealed that GHVIN1-derived hexose signalling influences cotton fibre initiation, probably through regulating transcription of several MYB transcription factors and auxin signalling genes required for fibre initiation. Together, our study provides an unprecedented example demonstrating that VIN-mediated hexose signalling could act as an early event modulating expression of regulatory genes, and hence cell differentiation in the ovule epidermis. A model describing the possible role of sugar signalling in trichome initiation will be discussed.
has been identified, the trait can be further replicated to other cereals for improved community health. Furthermore, since the responsible gene modification for delivering health-promoting nutrient-rich staple cereals for improved nutrition: nutritional enhancement of rice by thick aleurone phenotype

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Aleurone is rich in many important nutrients. With less than 10% of the total seed mass, aleurone is abundant with important nutrients ranging from lipid, minerals, vitamins and antioxidants. The large amount of valuable nutrients in this thin layer of cells makes aleurone the appropriate target for nutritional enhancement. The rice mutant ta2 (thick aleurone 2), isolated at the Institute of Botany, Chinese Academy of Science, was shown to have six to seven cell layers thick aleurone compared to the usual one cell layer. Detailed analysis of field grown wholegrain flour of the ta2 mutant demonstrated a general increment in most of the aleurone-enriched nutrients including lipid, minerals, B vitamins, antioxidants and phytylate. The increased mass of aleurone cells in the mutant retains aleurone identity, repressing the thicker 23a2 a larger sink of aleurone-associated nutrients and micronutrients and thus bringing significant increment in the nutritional value. The ta2 locus has been identified in the intron of a gene in chromosome 1, and this resulted in RNA alternative splicing and distortion of encoded protein sequence. The significance of this research lies on the indication that the thick aleurone trait has a high potential as a novel biofortification strategy for delivering health-promoting nutrient-rich staple cereals for improved community health. Furthermore, since the responsible gene modification has been identified, the trait can be further replicated to other cereals such as wheat where wholegrain consumption is substantial.

SYM-40-05

SMALL CHANGE IN SEED, BIG BENEFITS TO NUTRITION: NUTRITIONAL ENHANCEMENT OF RICE BY THICK ALEURONE PHENOTYPE

SYM-41-02

IDENTIFYING NOVEL SALINITY AND DROUGHT TOLERANCE MECHANISMS IN CEREALS

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We are facing the challenging task to meet the growing demand for food which must occur in an environment of a changing climate with increasing environmental stresses such as drought, extreme temperatures, nutrient deficiencies and mineral toxicities. Here, we are using metabolomics to improve our understanding of the mechanism(s) used by plants to either tolerate or to adapt to different abiotic stresses. We utilize GC-MS and LC-MS to define metabolic differences in a doubled haploid (DH) population from a cross between drought-intolerant (Kukri) and -tolerant (Excalibur) wheat cultivars grown in the field under a severe drought. Genetic linkage maps and marker scores enabled us to map QTL onto the wheat genome. In addition, we aim to develop and apply new tools to unravel how plants respond to the perception of salt stress. Evidence is accumulating that lipid signaling is an integral part of the complex regulatory networks in the responses of plants to salinity. We are using modern lipidomics technologies to compare the root plasma membrane (PM) compositions of different barley genotypes with contrasting salinity tolerance levels upon salt stress. Our aim is to investigate the link between PM composition and functionality in aspects of salinity response by examining whether observed changes in lipids are involved in either the alteration of fluid flow or in lipid-based downstream signaling. We are also using MALDI-FT-MS based imaging technologies to monitor spatial distributions of lipids across root sections of salt treated tolerant and intolerant barley genotypes. These novel findings will lead to a better understanding of the role of lipids, lipid composition and signaling for plant salt tolerance.

SYM-41-03

REPRODUCTIVE STAGE ABIOTIC STRESS TOLERANCE IN WHEAT

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Maintaining grain productivity under adverse weather conditions is an urgent requirement for important cereal crops such as wheat. The reproductive stage is very sensitive to abiotic stresses that have been predicted to become more prevalent under climate change conditions: drought, heat and cold/frost. Sterility (loss in grain number) is a phenotype that is shared by drought, shading, heat and cold conditions, suggesting that the abiotic stress response pathways leading to abortion of grain development under those different stress conditions may be shared. The young microspore stage of pollen development is the most sensitive stage to all these stresses and in self-pollinating cereals such as wheat, pollen sterility leads to reduced grain number. A controlled environment phenotyping method targeting this stage of high sensitivity was established to identify wheat germplasm that maintains fertility and grain production under drought, shading, heat and cold conditions. The drought-tolerant germplasm we identified also showed good tolerance to the other abiotic stresses (shading, heat and cold), providing further support to the hypothesis that the stress tolerance mechanism for maintaining pollen fertility may be shared by different stresses. We are currently in the progress of identifying the stress tolerance QTLs that overlap between the different stress treatments. Studies of the molecular and physiological basis provided evidence that germplasm with tolerance to different abiotic stresses also shared a similar hormonal response strategy. The project will provide opportunities for improving reproductive stage abiotic stress tolerance in wheat through marker-assisted breeding and the identification of candidate tolerance genes will further improve our understanding of the underlying molecular and physiological mechanism.

SYM-41-01

ETHYLENE PRIMING: A NEW MECHANISM THAT CONFERS FLOODING TOLERANCE

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The severity and frequency of floods is predicted to increase with changing global weather patterns. These floods adversely affect crop growth and yields. Research aimed at increasing plant resistance to abiotic stresses such as flooding is therefore a high global priority. The volatile plant hormone ethylene accumulates in submerged plant tissues very quickly (within 1 h) and acts as a reliable proxy for submergence and probably therefore this hormone operates upstream of several adaptive processes. In Arabidopsis, ethylene can prepare (prime) for flooding tolerance to later occurring oxygen deficiency (anoxia). This novel mechanism of flooding tolerance is regulated via stabilisation of group VII Methionine-Cysteine (MC) Ethylene Response Factor (ERF) transcription factors. How these proteins accumulate under normoxic conditions in ethylene pretreated tissues will be discussed.
SYM-41-04
EVALUATING CONTRIBUTION OF IONIC, OSMOTIC AND OXIDATIVE STRESS COMPONENTS TOWARDS SALINITY TOLERANCE IN BARLEY

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Abstract Background: Salinity tolerance is a physiologically multi-faceted trait attributed to multiple mechanisms. Three barley (Hordeum vulgare) varieties contrasting in their salinity tolerance were used to assess the relative contribution of ionic, osmotic and oxidative stress components towards overall salinity stress tolerance in this species, both at the whole-plant and cellular levels. In addition, transcriptional changes in the gene expression profile were studied for key genes mediating plant ionic and oxidative homeostasis (NHX; RBOH; SOD; AHA and GORK), to compare a contribution of transcriptional and post-translational factors towards the specific components of salinity tolerance. Results: Our major findings are two-fold. First, plant tissue tolerance was a dominating component that has determined the overall plant responses to salinity, with root K+ retention ability and reduced sensitivity to stress-induced hydroxyl radical production being the main contributing tolerance mechanisms. Second, it was not possible to infer which cultivars were salinity tolerant based solely on expression profiling of candidate genes at one specific time point. For the genes studied and the time point selected that transcriptional changes in the expression of these specific genes had a small role for barley’s adaptive responses to salinity. Conclusions: For better tissue tolerance, sodium sequestration, potassium retention and resistance to oxidative stress all appeared to be crucial. Because these traits are highly interrelated, it is suggested that a major progress in crop breeding for salinity tolerance can be achieved only if these complementary traits are targeted at the same time. This study also highlights the essentiality of post translational modifications in plant adaptive responses to salinity. Keywords: Stomatal conductance, Sodium sequestration, Potassium retention, Membrane potential, Tissue specific responses, H+-ATPase, Reactive oxygen species, Cytosolic ion homeostasis.

SYM-41-05
CLIMATE-INDUCED MANGANESE TOXICITY STRESS VS EVOLVED TOLERANCE: A STUDY OF TWO CONTRASTING PLANT ECOSYSTEMS

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The overaccumulation of foliar-manganese (Mn) and its associated impact on certain North American maple species has been attributed in part at least to changing climate variables. Artefactually induced foliar-Mn accumulation is recognised as one of several interactive factors whose net effects drive physiological stress, nutritional imbalance and heightened susceptibility to pest attack. This contrasts markedly with eastern Australian ecosystems in which naturally Mn-enriched substrates support inherently tolerant plants, some capable of scavenging and accumulating extraordinarily excessive foliar Mn concentrations that possibly even contribute to chemical defence against insect herbivory. Our study compares these two very different systems at the ecological and physiological levels.
SYM-42-03

IN SITU IDENTIFICATION OF BIPOTENT STEM CELLS IN THE MAMMARY GLAND

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The mammary epithelium undergoes profound morphogenetic changes during development. Architecturally, it comprises two primary lineages, the inner luminal and outer myoepithelial cell layers. Two opposing concepts on the nature of mammary stem cells (MaSCs) in the postnatal gland have emerged. One model, based on classical transplantation assays, postulates that bipotent MaSCs have a key role in coordinating ductal epithelial expansion and maintenance in the adult gland, whereas the second model proposes that only unipotent MaSCs identified by lineage tracing contribute to these processes. Through clonal cell-fate mapping studies using a stochastic multicolour cre reporter combined with a new three-dimensional imaging strategy, we provide evidence for the existence of bipotent MaSCs as well as distinct long-lived progenitor cells. The cellular dynamics at different developmental stages support a model in which both stem and progenitor cells drive morphogenesis during puberty whereas bipotent MaSCs coordinate ductal homeostasis and remodelling of the adult gland (Rios et al. 2014, Nature).

SYM-42-05

RNA-SEQ IDENTIFIES THE ZINC FINGER GENE, ZNF385B, AS A NOVEL PLAYER IN CHICKEN GONADAL SEX DIFFERENTIATION

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The embryonic gonads represent an ideal model for studying morphogenesis since they have a developmental choice: ovary or testis formation. Much progress has been made in recent years in understanding how these divergent pathways are genetically activated, regulated and maintained. However, gaps in our understanding remain. We carried out RNA-seq on early chicken embryos to seek novel players in gonadal sex differentiation. A number of genes were identified with sexually dimorphic expression, implicating them in ovary versus testis development. One such gene is ZNF385B, which encodes a zinc finger transcription factor with no previous link to gonadal development. This gene has a very restricted expression profile in the early chicken embryo, being expressed in the gonads, notochord, dermomyotome and the apical ectodermal ridge (AER) of the limb. In the gonads, ZNF385B is more highly expressed in males, where transcripts localise to the critical Sertoli cell lineage, with expression peaking during the onset of Sertoli cell organisation. This presentation will describe the functional analysis of ZNF385B. Experimental sex reversal of ovaries into testis using oestrogen blockers causes up-regulation of ZNF385B mRNA expression in females. We also report viral-mediated over-expression and analyse ZNF385B expression following knockdown of the critical male-determining gene, DMRT1, in male gonads. The data suggest that ZNF385B is a novel player in embryonic testis development in the avian model.

SYM-42-04

THE ZIC2 GENE MEDIATES CORRECT MIGRATION OF ANTERIOR NOTOCHORD PRECURSORS TO PREVENT HOLOPROSENCEPHALY

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Holoprosencephaly (HPE) is a severe congenital disease, affecting up to 1/250 pregnancies, in which failed dorso-ventral (D-V) forebrain patterning by a structure called the prechordal plate (PCP) prevents hemispheric separation. Mutation of ZIC2, a member of the ZIC gene family of multifunctional transcriptional regulators, has been shown to produce HPE in humans. Furthermore the HPE phenotype is recapitulated in the kumba mouse strain via induction of a severe loss of function mutation in the murine Zic2 homologue. Kumba homozygotes show normal formation of the PCP initially, however due to a transient defect in node at midgastrulation, the PCP degrades. Epiblast cells ingressing through the node at midgastrulation normally undergo differentiation to become the axial mesoderm of the anterior notochord (ANC), which then provides a maintenance signal to the PCP. In kumba homozygotes however, the defective node fails to produce adequate ANC cells, the PCP subsequently degrades and therefore is not present to mediate D-V patterning during neurulation. The dearth of ANC cells is shown to be unrelated to either apoptosis or proliferation defects, therefore we investigated the hypothesis that ANC precursors may undergo incorrect migration. A carboxycyanine dye, Dil, was injected into the node of embryos at the late streak stage of gastrulation, and these embryos then cultured for 24 hours in vitro. Examination of kumba homozygous embryos revealed aberrant migration of ANC precursors along the embryonic midline. We propose that this defect of migration is the mechanism by which ANC development is compromised in the context of Zic2-dependent HPE.

SYM-43-01

THE PRENUCLEOSOME, A NOVEL NONNUCLEOSOMAL HISTONE-DNA PARTICLE

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DNA in the eukaryotic nucleus is packaged into a nucleoprotein complex termed chromatin. The standard unit of chromatin is the nucleosome, which, in metazoans, typically comprises the core histone octamer, one molecule of histone H1, and about 180-200 bp of DNA. It is important to note, however, that chromatin is dynamic and diverse, and thus contains both canonical nucleosomes as well as nonnucleosomal histone-DNA structures that are likely to be important constituents of active chromatin. We recently discovered a novel nonnucleosomal histone-DNA intermediate in the assembly of chromatin, which we termed this particle as the “prenucleosome” because it is a precursor to the nucleosome. Importantly, prenucleosomes satisfy the criteria for a reaction intermediate - they can be converted into nucleosome arrays at a rate that is comparable to that of the overall assembly reaction. By atomic force microscopy, prenucleosomes are indistinguishable from conventional nucleosomes. The existence of prenucleosomes provides a resolution to an old paradox that goes back to the 1970s. At that time, it was observed that nucleosome-like structures (containing at least histones H3 and H2B) form within seconds upon passage of DNA replication forks, whereas canonical “mature” nucleosomes, as characterized by nuclease digestion and sedimentation properties, are more slowly generated after approximately 10 to 20 min. Thus, the question was - how could nucleosome-like particles be formed within seconds when it was also known that it takes at least 10 min to form a canonical nucleosome? It now appears that this paradox can be explained by the rapid formation of prenucleosomes at DNA replication forks. In the talk, I will discuss prenucleosomes as well as other aspects of chromatin assembly and dynamics.
**SYM-43-02**

**LINKING EPIGENOMICS TO BIOLOGY: INSIGHTS FROM DEVELOPMENTAL CANALISATION IN HONEY BEES**

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Epigenomics deals with chemical decorations such as DNA methylation or abundant chromatin proteins modifications and their involvement in tissue-specific and context-dependent gene expression. These versatile components of gene regulatory networks act as prime drivers of organisational complexity, robustness, plasticity and disease development. We proposed that by providing organisms with a large repertoire of alternative functional interactions, the epigenomic modifications increase their adaptability to unforeseen environments. But how do we evaluate what these chemical modifications mean in a functional context? How environment is linked to the genome and how external cues are translated into cellular responses via epigenomic changes? When do these changes go above threshold and guide organisms into another direction, such as an alternate developmental trajectory? How does malfunctioning of epigenetic mechanisms result in diseases? To what extent sequence variants such as single nucleotide polymorphism (SNPs) affect epigenomic marks? This is an area in which excellent progress has been made using the honey bee, *Apis mellifera*, an emerging system to investigate epigenomes in both developmental and behavioural contexts. Worker bees use an environmental cue (royal jelly) to de-canalise female larval development in order to generate two contrasting organisational outcomes: one fertile, long-lived with a large body mass (queen), the other sterile, short-lived with lower body mass and phenotype-specific organs (worker). This attractive example of developmental flexibility nicely illustrates how environmental factors can control an organism's genetic hardware to yield context-dependent outcomes, both anticipated and detrimental.


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**SYM-43-03**

**EPIGENOME ENGINEERING IN CANCER**

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With the recent comprehensive mapping of cancer genomes, there is now a need for functional approaches to edit the aberrant epigenetic state of key cancer drivers to reprogram the epigen-pathology of the disease. In breast cancer, tumor suppressor genes, such as mammary serine protease inhibitor (MASPIN) are more frequently inactivated by epigenetic mutations than by genetic mutations. In contrast, oncogenic drivers such as SOX2, MYC and FOXM1 are frequently amplified and overexpressed in breast cancer. Our laboratory has developed epigenomic tools to re-write the specific epigenetic modifications controlling the expression of these genes. We have generated programmable DNA-binding proteins that recruit epigenetic modifiers to either reactivate hypermethylated tumour suppressor genes or stably silence oncogenic gene expression. We outline a novel genomic approach to induce-long lasting tumour growth inhibition with potential applicability to many cancer drivers currently refractory to drug design.

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**SYM-43-04**

**CHANGES IN DNA ACCESSIBILITY OCCUR IN PUTATIVE ENHANCER REGIONS OF A SUBSET OF T CELL MEMORY RESPONSIVE GENES**

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Naive T cells exist in a resting state until activated by the antigen they are specific for. Upon activation, T cells produce cytokines and other proteins necessary to fight infections and proliferate to produce more T cells specific for that antigen. Some of these descendants will be long-lived memory T cells, which will return to a resting state but are primed for a more rapid and robust immune response upon re-exposure to the same antigen. The molecular mechanisms responsible for this enhanced response are still unclear. Using a T lymphocyte cell culture model of transcriptional memory we have investigated the possibility that previously stimulated cells have differences in DNA accessibility compared to non-stimulated cells. We used Formaldehyde-Assisted Isolation of Regulatory Elements (FAIRE) in conjunction with high throughput sequencing to identify changes in DNA accessibility in response to stimulation, resting and re-stimulation. We found that although many of the changes in accessibility that were induced upon stimulation, were reversed upon stimulus withdrawal and resting of the cells, a large proportion remain accessible and a subset of these are near genes that have enhanced induction upon re-stimulation. Interestingly the accessibility associated with these ‘memory-responsive’ genes was not always at the transcription start site but sometimes occurred in putative enhancers of these genes. We are currently characterising these regions and examining their potential role in regulating gene induction in memory T cells.

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**SYM-43-05**

**PROBING THE STRUCTURE AND FUNCTION OF A 1-MDA CHROMATIN REMODELING COMPLEX**

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The fact that DNA-binding transcription factors bind to specific sites in gene promoters and thereby regulate the expression of target genes is well established. However, at a molecular level, the steps between DNA binding and changes in the expression levels of target genes are only partly understood, and the goal of our work is understand at a mechanistic level the changes in chromatin structure and occupancy and the protein interactions that drive these changes in gene expression. We are currently working to determine the structure of the Nucleosome Remodeling and Deacetylase (NuRD) complex – a 10-subunit, ~1-MDa transcriptional coregulator that is essential for normal growth and development across all complex organisms. This talk will describe single-particle negative-stain and cryo-electron microscopy data on the NuRD complex, and the integration of these data with other structural and mass spectrometry data to create the first model of a mammalian gene coregulatory complex of this type. These data provide a glimpse into the mechanisms through which complex coregulator complexes are recruited to target genes and help to map out the molecular events that drive gene regulation.
SYM-44-01

SINGLE-MOLECULE IMAGING OF CELLULAR ASSEMBLY AND DISASSEMBLY PROCESSES

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A remarkable feature of cellular systems is the emergence of higher order structures from the stochastic motions and interactions of individual molecules. Molecular machines play a key role in controlling these interactions and ensure that protein complexes assemble and disassemble at the right time and the right place in the cell. The machinery responsible for maintaining homeostasis of the entire proteome is the network of molecular chaperones. Age-related decline of chaperone function results in protein misfolding, aggregation and the onset of severe neurodegenerative diseases such as Huntington’s and Parkinson’s disease. Members of the heat shock protein 70 (Hsp70) family are central players in the chaperone network. Hsp70 is an ATP driven molecular machine that clamps onto unstructured, hydrophobic regions in proteins. Hsp70 family members bind a broad range of substrates and are astonishingly versatile. Apart from the basic function of protein folding, they play crucial roles in the assembly and disassembly of macromolecular structures. Given this broad substrate specificity, there are over 40 co-chaperones in mammalian cells that recruit Hsp70 to a specific task at the appropriate time. Here we use single-molecule imaging in conjunction with ensemble biochemical methods to address the mechanisms used by these molecular machines to catalyse the restructuring of macromolecular complexes.

SYM-44-02

PRIONS HERE, PRIONS THERE, PRIONS EVERYWHERE

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Here we will focus on the subset of proteins forming fibrils and oligomers that have the fascinating ability to propagate and trigger aggregation of other proteins. We will describe a rapid way to characterize interactions and aggregation of these proteins in vitro using a combination of eukaryotic cell-free protein expression, AlphaScreen assay for protein-protein interactions and single molecule fluorescence. In general, single molecule fluorescence detects easily oligomers and we will discuss a novel analysis of burst brightness enabling rapid diagnostic of protein behavior. We combined this with AlphaScreen, a very sensitive binding assay detecting interactions between two nanobeads. Highly multiplexable, AlphaScreen is used to rapidly screen for binding partners. The hits are further characterized at the single molecule level, where two-color coincidence experiments determine if the binding occurs at the monomer level or in the aggregated form. This set of technique was applied to multiple proteins involved in neurodegenerative disorders. Unexpectedly, the same prion behavior was observed in completely unrelated systems, especially in proteins of the immunity pathways and we will discuss the biological implications of such phenomenon.

SYM-44-03

MALARIA VIRULENCE: A SUPER RESOLUTION SOLUTION

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Survival of the human malaria parasite Plasmodium falciparum in the circulation of the host relies on its ability to drastically alter its red blood cell (RBC) host cell. This remodelling is mediated by the export of parasite-derived proteins such as the major knob protein knob associated his-rich protein, which interacts with components of the RBC cytoskeleton and modify RBC behaviour. We functionally assay the RBC cytoskeleton remodelling processes across both asexual and sexual blood stage (Gametocyte) development, by combining parasite reverse genetics with ektocytometry, spleen mimic filtration assays, super resolution microscopy and live cell microscopy techniques. We show that the asexual stage parasites and early stage gametocytes employ different mechanisms to mediate their RBC deformability, with the early stage gametocyte exhibiting a highly undeformable RBC in the absence of many of the known cytoskeleton interacting proteins, indicating that modulation of the host cell cytoskeleton itself may contribute to RBC rigidification. In addition gametocyte specific exported proteins play an important role in this remodelling process. We show that these RBC modifications of the early stage gametocyte are reversed in the late stage gametocyte allowing parasite survival within the host and disease transmission.

SYM-44-04

ELUCIDATION OF T CELL FATE CONTROL BY TIME LAPSE IMAGING AND QUANTITATIVE MICROSCOPY

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Until recently, studies of immune cell fate determination have utilized population-based approaches, making the behavior of minority and/or transient cell types difficult to capture. We have recently developed new methods to quantify behavior of single cells using high throughput fluorescence time lapse microscopy, and have used these approaches to make seminal findings related to the acquisition of memory and effector attributes in CD8+ T cells. We derived 10-generation pedigrees of individual naïve CD8+ cells responding to antigen presentation in vitro, and used these pedigrees to elucidate novel parameters controlling proliferation, apoptosis and effector/memory differentiation. We see a broad heterogeneity in the response of individual naïve T cells that matches the heterogeneity recently described in vivo. This heterogeneity is mostly restricted to the founder cell in each pedigree, with most pedigrees exhibiting strong sibling correlations. Some of the pedigrees do show heterogeneity amongst the progeny, but this is predominantly restricted to the first daughter cells, with subsequent progeny again showing strong correlations. These findings provide novel insight into the relative role of intrinsic, extrinsic and stochastic factors in regulating CD8 T cell fate.

ComBio2014 • Canberra, ACT, Australia • 28 September - 2 October, 2014 Page 79
Symposia

Thursday

Sym-44-05

STRUCTURAL REARRANGEMENT OF HIV-1 PRIOR TO UNCOATING REVEALED BY SINGLE-MOLECULE LOCALISATION MICROSCOPY

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Infection of human T lymphocytes by the human immunodeficiency virus-1 (HIV-1) begins with the binding of viral envelope (Env) proteins to cell surface receptor CD4 and chemokine co-receptor (CXCR4 or CCR5), which mediate virus entry. Following fusion to the membrane, the “cone-shaped” viral capsid is released into the cytoplasm and undergoes an uncoating process to expose the viral RNA. Although the size and the shape of the capsid are assumed to be stable during the fusion process, little is known about the morphology of the virus prior to the release of the capsid or whether receptor binding would facilitate the delivery of the genetic material into the cell. Conventional light microscopes are limited to a resolution of 250 nm, and therefore knowledge about events occurring at the subviral scale is mainly based on electron microscopy data and visualisation of pre-fusion events has yet to be performed. The recent development of super-resolution microscopy techniques and their applications in biology have allowed us to combine nanoscale resolution and the versatility of fluorescence microscopy. Using single-molecule localisation microscopy (i.e dSTORM), electron microscopy and morphological analysis, we were able to visualise the viral particle, matrix and capsid and to determine their size and morphology upon CD4 engagement. We observed that the interaction between CD4 molecules and an HIV-1 particle is sufficient to initiate an expansion of both the HIV particle and the capsid core. Our insights into the organisation of HIV-1 at the nanoscopic scale suggest a remodelling of the internal architecture of the virus upon Env engagement prior to the disassembly of the HIV-1 core.

Sym-45-01

A NON-CANONICAL N-END RULE PATHWAY OF PROTEIN DEGRADATION IN MITOCHONDRIA

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Intracellular protein degradation is a regulated process responsible for the removal of unwanted or damaged proteins for regulatory purposes and protein quality control. It is mediated by ATP-driven enzymes such as the 26S proteasome and Clp proteases. One highly conserved mechanism of protein degradation is the N-end rule pathway. Proteins bearing a destabilising amino acid residue at their N-terminus are targeted for degradation by the ClpAP protease in bacteria or by the 26S proteasome in the eukaryotic cytosol. In bacteria exposure of N-terminal Leu, Phe Tyr and Trp, renders the protein highly susceptible to ClpAP mediated degradation. To date a mitochondrial N-end rule pathway of protein degradation has not been described. However, recently a new post-translational modification, known as intermediate cleaving peptidase 55 (iCp55), was identified in yeast. This exopeptidase cleaves a single hydrophobic or aromatic residue from the N-terminus of a subset of mitochondrial proteins following removal of the mitochondrial targeting signal by mitochondrial processing peptidase. Loss of this pathway, through a post-translational modification, by iCp55, renders the processing intermediate unstable. Since these unstable intermediates expose an N-terminal Tyr, Leu or Phe, it has been proposed that an N-end rule protein degradation pathway also operates in mitochondria. Here we describe the components of this pathway in mammalian mitochondria and show that the processing intermediates are degraded by a non-canonical N-end rule pathway.

Sym-45-02

DEFINING THE ROLE OF E3 UBIQUITIN LIGASE UBR5 IN CANCER THROUGH SUBSTRATE IDENTIFICATION

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Identification of E3 ubiquitin ligase substrates is key to defining their biological function and understanding their roles in disease. However, even with advances in proteomics and in vitro assays, substrate identification remains a significant challenge. We have developed an integrated approach define the substrates of the E3 ligase UBR5, combining orthogonal proteomics approaches to identify UBR5-interacting proteins and ubiquitylation targets, combined with high-throughput BiFC to validate substrates in situ. Altered expression and somatic mutations of UBR5 have been observed in numerous cancer types, and UBR5 expression modulates chemoresistance in ovarian cancer, likely through regulation of the DNA damage response. However, substrates of UBR5 are not well characterised. UBR5-interacting proteins were isolated using GFP-Trap affinity purification followed by nanoLC-MS/MS identification and label-free quantitation. We identified 198 putative high-confidence UBR5-interacting proteins and analysis of differentially ubiquitylated proteins in breast cancer cells depleted of UBR5 by shRNA identified a number of putative substrates that are also UBR5 interacting proteins. We have validated a number of these hits using BiFC and have mapped the role of UBR5 functional domains in mediating these interactions using disease-specific mutants. These orthogonal but complementary approaches are providing interesting new insights into the function UBR5, suggesting a role in mediating crosstalk between DNA damage response and transcriptional regulation.

Sym-45-03

REGULATING IRON TRANSPORT THROUGH UBIQUITINATION

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Ubiqution is a major mechanism of regulating membrane proteins, such as signalling receptors, ion channels and transporters. Ubiqution tagging acts as a signal to provide a mechanism for internalization, intracellular sorting and degradation of these membrane proteins. In many cases the ubiquitination mediated by the members of the Nedd4 family of ubiquitin ligases (E3s) regulates membrane protein, including those involved in sodium homeostasis, such as the epithelial sodium channels and voltage-gated sodium channels. These channels directly bind the WW domains in Nedd4-2, a member of the Nedd4 family, through the PPxY motifs present in their C-termini. We discovered adaptor protein Ndfip1 and Ndfip2 which appear to facilitate binding between Nedd4 E3s and substrates that lack PPxY motifs. One such substrate is DMT1, the primary non-heme iron transporter in mammals and our work described a novel mechanism of DMT1 regulation involving Ndfip1 and WWP2 (a Nedd4 family member), where ubiquitination controls iron homeostasis via the degradation of DMT1. Our more recent studies now identify α-arrestins Arrdc1 and Arrdc4 as new adaptors for Nedd4 E3s, which are involved in the regulation of DMT1. Unlike the Ndfips, ArrdcS decrease DMT1 ubiquitination, and instead of mediating DMT1 endocytosis, they appear to promote DMT1 release in membrane vesicles. Thus, through different adaptors the Nedd4 E3s can regulate ubiquitin-dependent regulation of iron transport through novel mechanisms.
SYM-46-01

ESTIMATING TURNOVER TIME OF LEAF WATER POOLS USING STABLE ISOTOPES

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A knowledge gap in leaf hydraulics exists between liquid water entering the lamina tissue from the xylem and vapour exiting the leaf through the stomata. We don’t know the path (or paths) water takes to reach the sites of phase change and we don’t know where these evaporating sites are in the leaf. Measurements of stable isotopes of water, both vapour and liquid, may help to address these gaps. Using coupled leaf gas exchange and online measurements of the isotopic composition of water vapour, we calculated the turnover time of cotton leaves in a range of evaporative environments. The time constant for turnover varied between 60 and 107 minutes, increasing under conditions of low transpiration rate as expected. A model with a single leaf water pool best reproduced the timing and rate of change in δ¹⁸O of the transpiration stream in cotton. Measurements of δ¹⁸O of transpired vapour were also used to calculate the ³²⁰O enrichment at the sites of evaporation within the leaves (Δe), and compared to enrichment of water extracted from the bulk leaf tissue (Δt). The fractional difference between Δe and Δt did not increase with increasing transpiration rate, and was not related to the difference between stem and leaf water potential. The data do not support a radial Peclet effect within leaves, but rather suggest that a proportion of leaf water (18%, on average) is not evaporatively enriched. Taken together, these measurements imply two pools with very similar turnover times.

SYM-46-04

EPITHELIAL CELL MOVEMENT AND THE NUCLEAR AND CYTOPLASMIC FORMS OF THE ESSENTIAL PROTEASE DIPETIDYL PEPTIDASE-9

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The intracellular protease dipetidyl peptidase 9 (DPP9) can influence cell adhesion and migration, and cell proliferation via the EGF/Pi3K/Akt pathway, and can increase cell apoptosis. In EGF simulated hepatoma cells, some DPP9 was localised in ruffling cell membranes, inside the leading edge of migrating cells. At focal adhesions, punctate DPP9 was co-localized with β1-integrin and talin. DPP9 siRNA knockdown and a DPP9 inhibitor impaired in vitro cell adhesion and migration and reduced β1-integrin and talin levels and the phosphorylation of both focal adhesion kinase and paxillin. By confocal and super resolution microscopy, we localized DPP9 to most intracellular structures, including mitochondria, microtubules and nucleus. Homozygotes of a mouse strain we made that lacks DPP9 catalytic activity die soon after birth. Many cell proliferation and cell activation genes were differentially expressed in epithelial organs of these mice. The tensile strength of heterozygote adult skin was less than that of wild type littermates. We identified 56 potential DPP9 substrates in nuclear and cytoplasmic extracts of cells from these mice using 2D-Difference gel electrophoresis and MALDI TOF MS. Several of these substrates are significant in immunology, cancer biology and energy metabolism and were confirmed as substrates by digestions of synthetic peptides with purified human DPP9. DPP9 is in epithelial cells and upregulated in tumour cells and activated lymphoid cells. Our data provides mechanistic insights into roles of DPP9 in intracellular signaling and cell movement in such cells.

SYM-45-04

OSMOTIC ADJUSTMENT AND TURGOR MAINTENANCE IN SALT-STRESSED WHEAT AND BARLEY

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Mechanisms for salt tolerance in crop species fall into three main categories: 1) tolerance to the osmotic stress caused by salts in the soil solution, 2) reducing salt entry and build up in leaves through salt exclusion processes, and 3) the minimization of salt build up in the cytoplasm through efficient vacuolar compartmentation. Efficient osmotic adjustment and turgor maintenance play a unique role in contributing to overall salt tolerance via each of these mechanisms. This is illustrated by the differences in salt tolerance between three related species: durum wheat, bread wheat and barley, where only barley has the ability to accommodate high concentrations of Na and Cl in leaves over extended periods of time. Efficient osmotic adjustment enables barley to make use of the salts in the soil, a trait normally shown by halophytes.

SYM-45-05

FIBROBLAST ACTIVATION PROTEIN (FAP) DEGRADOMICS HIGHLIGHTS THE ROLE OF FAP IN EXTRACELLULAR MATRIX

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Fibroblast Activation Protein (FAP) expression is predominantly associated with disease states, including cancer, liver and lung fibrosis, arthritis and atherosclerosis. FAP is a cell-surface anchored dimeric protease and glycoprotein that is closely related to Dipetidyl Peptidase IV (DPP-IV / DPP4). The few known FAP substrates include collagen I. This study aims to identify novel FAP substrates and downstream effects of FAP enzyme activity on the proteome. Primary mouse embryonic fibroblasts (MEFs) were isolated from FAP gene knockout mice. These cells were immortalised by transduction with the SV40 Large T antigen. FAP and GFP were expressed in an inducible expression system. A second construct that was FAP enzyme activity-inactive contained a Ser to Ala substitution at the catalytic site [Ser624]. To identify substrates, Terminal Amine Isotopic Labelling of Substrates (TAILS) was employed to analyse the secretome of MEF cell lines expressing functional enzyme (FAP e+) vs inactive FAP (FAP e-). Many post-proline cleavage sites after Gly-Pro in collagen were observed, consistent with known FAP specificity, which was confirmed by PICS analysis. Post-proline cleavage of a number of small bioactive peptides and other ECM proteins was also observed. In addition, the impact of FAP activity on secretome composition was investigated using SILAC in a quantitative proteomic strategy. Proteins identified with altered abundance were predominantly associated with wound healing and with the extracellular matrix. Consistent with the concept of an interconnected Protease Web, several proteases displayed differential abundance. Preliminary studies of in vitro wound healing found that FAP e+ MEF cell lines exhibited greater cell migration but exhibited comparable proliferation and apoptosis to FAP e- MEF cells. These data provide insights into the biological functions of FAP by elucidating its substrates and the global cellular effects of ablating its enzyme activity.
Aquaporins are proposed to have a major role in growth of plants via their impact on root water uptake, growth and leaf gas exchange. Under saline conditions growth is depressed due to osmotic stress and salt can accumulate in leaves leading to further depression of growth due to reduced photosynthesis and gas exchange. The aquaporin, GmPIP1;6 is associated with control of root hydraulic conductance (Lo) in soybean, and is highly expressed in roots as well as reproductive tissues. It is a functional aquaporin when expressed in Xenopus oocytes and is targeted to the plasma membrane in onion epidermis. Treatment with 100 mM NaCl resulted in reduced expression initially then after 3 days the expression was increased. The effects of constitutive overexpression of GmPIP1;6 in soybean was examined under normal and salt stress conditions. Overexpression in 2 independent lines resulted in enhanced leaf gas exchange, but not growth under normal conditions compared to wild type (WT). With 100 mM NaCl, net assimilation was much higher in the GmPIP1;6-Overexpression line than in WT. GmPIP1;6-Overexpression plants did not have higher root hydraulic conductance (Lo) under normal conditions, but were able to maintain Lo under saline conditions compared to WT which decreased Lo by a factor of about 2. GmPIP1;6-Overexpression lines grown in the field had increased yield resulting mainly from increased seed size. The general impact of overexpression of GmPIP1;6 suggests that it may be a multifunctional aquaporin involved in root water transport, photosynthesis and seed loading. In addition to GmPIP1;6, we also analyzed the expression pattern and function of GmPIP2;9 by transforming constructs of native PIP2;9 promoter driving Gus reporter and 3SS promoter driving PIP2;9 cDNA. Primary data showed that overexpression of GmPIP2;9 led to an enhanced drought tolerance in transgenic soybean.

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**SYM-46-04**

**A SHIFT OF DRIVING FORCE FOR WATER UPTAKE INTO GRAPE BERRIES DURING THE COURSE OF RIPENING**

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Grape berry growth is largely determined by the ratio between water uptake and water loss from fruits during berry development. Pre-harvest berry dehydration, a net water loss from berries at the end of ripening, is a serious problem causing shrivelling of fruits and concentration of sugars making affected berries less desirable for wine production. In Australia, the most commonly grown red grape variety Vitis vinifera L. cv. Shiraz is particularly susceptible to this disorder. The current hypothesis is that this net water loss occurs due to a greater decrease in influx compared to water loss by transpiration during ripening. Using an in vitro transpiration assay for individual Grenache and Shiraz berries, we found that xylem water uptake into berries at early stages of development was sufficient to balance water loss via transpiration, but diminished at veraison resulting in a net water loss - which was more severe in berries of Shiraz compared to berries of Grenache. This is consistent with the hypothesis that the primary mechanism of grape berry water uptake switches from xylem to phloem water uptake at veraison. The rapid decline of xylem water uptake at veraison coincided with a significant drop in driving force for water uptake generated by transpiration. Hence, osmotically driven water uptake may be more important in post-veraison berries. A loss of cell membrane integrity, as reported for ripening berries of Shiraz, could cause a loss of driving force for water uptake resulting in the observed shrivelling.

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**SYM-46-05**

**CONSTITUTIVE OVEREXPRESSION OF SOYBEAN PLASMA MEMBRANE INTRINSIC PROTEIN GMP1P1;6, GMP1P2;9 CONFRS DROUGHT AND SALT TOLERANCE**

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Aquaporins are proposed to have a major role in growth of plants via their impact on root water uptake, growth and leaf gas exchange. Under saline conditions growth is depressed due to osmotic stress and salt can accumulate in leaves leading to further depression of growth due to reduced photosynthesis and gas exchange. The aquaporin, GmPIP1;6 is associated with control of root hydraulic conductance (Lo) in soybean, and is highly expressed in roots as well as reproductive tissues. It is a functional aquaporin when expressed in Xenopus oocytes and is targeted to the plasma membrane in onion epidermis. Treatment with 100 mM NaCl resulted in reduced expression initially then after 3 days the expression was increased. The effects of constitutive overexpression of GmPIP1;6 in soybean was examined under normal and salt stress conditions. Overexpression in 2 independent lines resulted in enhanced leaf gas exchange, but not growth under normal conditions compared to wild type (WT). With 100 mM NaCl, net assimilation was much higher in the GmPIP1;6-Overexpression line than in WT. GmPIP1;6-Overexpression plants did not have higher root hydraulic conductance (Lo) under normal conditions, but were able to maintain Lo under saline conditions compared to WT which decreased Lo by a factor of about 2. GmPIP1;6-Overexpression lines grown in the field had increased yield resulting mainly from increased seed size. The general impact of overexpression of GmPIP1;6 suggests that it may be a multifunctional aquaporin involved in root water transport, photosynthesis and seed loading. In addition to GmPIP1;6, we also analyzed the expression pattern and function of GmPIP2;9 by transforming constructs of native PIP2;9 promoter driving Gus reporter and 3SS promoter driving PIP2;9 cDNA. Primary data showed that overexpression of GmPIP2;9 led to an enhanced drought tolerance in transgenic soybean.

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**SYM-47-01**

**SONIC HEDGEHOG AND PRIMARY CILIA REGULATE A SWITCH IN AXON GUIDANCE RESPONSES IN THE SPINAL CORD**

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Axons of the developing nervous system must navigate accurately along stereotyped pathways, often over long distances, in order to find their appropriate synaptic target. Commissural axons of the spinal cord have provided an informative in vivo model to study axon pathfinding. These axons extend ventrally from the dorsal spinal cord and cross the floorplate at the ventral midline, before making a sharp rostral turn and growing longitudinally. Upon reaching their intermediate target (the floorplate), commissural axons switch their responsiveness from attraction to repulsion, via mechanisms that remain poorly defined. Here, I report that the floorplate-derived cue Sonic hedgehog (Shh) initiates transcriptional activity in the nucleus of commissural neurons, to induce the expression of guidance receptors required for a subsequent step in their navigation. Further, I show that primary cilium function is required for this process. Interestingly, this Shh-mediated pathway induces the expression of different receptors in mouse and chick. However, in both systems primary cilium function is required to trigger a repulsive response in post-crossing commissural axons.
Oligodendrocyte progenitor cells (OPCs) are unique amongst glial cells. During development and adulthood they receive direct synaptic input from neurons. These axon-OPC synapses are maintained as the OPC undergoes cell division and each daughter cell “inherits” synaptic connections from the parent OPC. Signaling via the axon-OPC synapse can promote myelination but how axon-OPC synaptogenesis is initiated and regulated and how this process influences oligodendrocyte differentiation and myelination is unclear. Our recent electrophysiology experiments have shown that the membrane properties of OPCs in vitro closely resemble those of OPCs in acute slices – they express voltage-gated sodium and potassium channels, but fail to initiate true action potentials. Time-lapse imaging of primary OPC cultures revealed that OPC processes are highly dynamic in vitro, and when co-cultured with neurons, the processes of OPC interact transiently with axons prior to myelination and express many of the scaffolding proteins associated with synaptogenesis. OPC processes bear a striking resemblance to axonal growth cones, showing a complex repertoire of lamellipodial and filopodial dynamics including veiling, extension and retraction. Using an in vitro motility assay, we determined that OPC processes are not attracted to a gradient of brain derived neurotrophic factor.

OPC processes are highly dynamic in vitro, and when co-cultured with neurons, the processes of OPC interact transiently with axons prior to myelination and express many of the scaffolding proteins associated with synaptogenesis. OPC processes bear a striking resemblance to axonal growth cones, showing a complex repertoire of lamellipodial and filopodial dynamics including veiling, extension and retraction. Using an in vitro motility assay, we determined that OPC processes are not attracted to a gradient of brain derived neurotrophic factor. However OPC processes showed increased veiling (27.3 ± 0.6 min) and extension (3.1 ± 2 μm/hr) in response to a gradient of glutamate (1 μmol/L) compared with vehicle (18.3 ± 1.3 min and ~4.9 ± 1.9 μm/hr respectively, n=14). We propose that changes in neural activity can effect OPC behaviors ranging from proliferation, to process motility, synaptogenesis and ultimately differentiation.

**SYM-47-03**

**MUTATIONS IN USP9X CAUSE INTELLECTUAL DISABILITY AND EFFECT NEURONAL MIGRATION AND AXON GROWTH**

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We recently identified three unique mutations in USP9X as a novel cause of intellectual disability (ID). USP9X encodes a substrate-specific deubiquitylating enzyme, however all three mutations alter the protein’s C-terminus of unknown function. To investigate the cellular mechanisms underlying ID we utilised our Usp9x conditional knockout mouse. Brain specific loss of Usp9x prior to neurogenesis disrupted cortix architecture and reduced hippocampal volume. Using ex-vivo neural stem and neuronal cell cultures, we further discovered defective differentiation, neuronal migration and axon growth. Whilst expression of wild-type human USP9X could rescue the neuronal cell defects, expression of the mutants could not. That the USP9X mutants behaved essentially as complete loss-of-functions in the neuronal cell assays encouraged us to use this cell model to interrogate the underpinning molecular mechanisms. Proteins known to bind the C-terminus of USP9X include Doublecortin, a microtubule associated protein (MAP), and Smurf1, a regulator of TGFβ signalling. Both proteins regulate cell migration and axon growth. Consistent with a disruption of Doublecortin function, proteomics analysis of neurons lacking Usp9x revealed altered expression of multiple tubulin subunits and MAPs. Furthermore, mutant USP9X failed to localise with Doublecortin in axonal growth cones. These same neurons also displayed altered responses to TGFβ signalling, thus revealing a second potential mechanism underlying the axonal growth defects. Our data suggest that USP9X mutations alter multiple, coordinated molecular pathways in neurons, leading to disruption of axon growth and migration that likely underpins aspects of the altered brain development in patients with ID.

**SYM-47-04**

**SUMOYLATION OF ZIC5 EXERTS MULTIPLE EFFECTS ON TRANSCRIPTION TO PROMOTE NEURAL CREST CELL DEVELOPMENT**

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The Zinc fingers of the cerebellum (Zic) proteins are multi-functional regulators of transcription critical for several aspects of embryogenesis including development of the neural crest, a transient population of cells that gives rise to an amazing variety of cell types. The Zic proteins act as classical transcription factors to bind DNA and promote transcription of the Foxd3 gene during neural crest cell development. In addition, they act as co-factors that bind Tcf molecules to inhibit Wnt/β-catenin dependent transcription without contacting DNA. Exactly how the molecular functions of Zic proteins combine to ensure timely activation of the neural crest specifier genes in response to Wnt instructive signals is unknown. Here we show the balance between transcription factor and co-factor function of the Zic5 protein is influenced by post-translational modification of a highly conserved lysine. SUMOylation of this lysine in response to canonical Wnt signalling confers maximal transactivation capability. Conversely, specific or global inhibition of SUMOylation promotes Tcf/Zic complex formation and confers optimal co-factor activity. This model implies that both Zic5 and Zic5-SUMO are critical for neural crest cell development and analysis of a mouse strain incapable of Zic5 SUMOylation confirms this prediction.

**SYM-47-05**

**THE ROLE OF THE NEURAL CELL ADHESION MOLECULE IN MATURATION OF SYNAPTIC CONTACTS**

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During brain development, synaptic contacts between neurons have to undergo a complex process of maturation to establish fully functional synapses. The neural cell adhesion molecule (NCAM) is a member of the immunoglobulin superfamily of cell adhesion molecules, which rapidly accumulate in nascent synapses within minutes after first contact formation. We demonstrate that in developing neurons NCAM promotes maturation of the molecular machinery responsible for recycling of synaptic vesicles, organelles which store and release neurotransmitters. NCAM interacts with and recruits to the presynaptic membrane the adaptor protein 2 (AP-2), a protein complex involved in clathrin-dependent synaptic vesicle endocytosis. Also in mature synapses, disruption of the NCAM-mediated synaptic adhesion results in reduced synaptic vesicle recycling and inhibits the ability of synapses to respond to the repetitive stimulation. Our results thus indicate that disruption of NCAM functions in humans may result in abnormalities in neurotransmitter release, which may contribute to aetiology of psychiatric disorders associated with mutations in NCAM gene.
Protein misfolding and aggregation as a consequence of impaired proteostasis not only characterizes numerous age-related diseases but also the aging process itself. We determined by iTRAQ proteomic analysis in the nematode Caenorhabditis elegans how the proteome changes with age and in response to heat shock. Levels of ribosomal proteins and mitochondrial chaperones were decreased in aged animals, supporting the notion that proteostasis is altered during aging. Mitochondrial enzymes were also reduced, consistent with an age-associated energy impairment. Moreover, we observed an age-associated decline in the heat shock response. In order to determine how protein synthesis is altered in aging, we complemented our global analysis by examining the de novo proteome. We established methods to visualise and identify de novo synthesised proteins in C. elegans, by incorporating the non-canonical methionine analogue, azidohomoalanine (AHA), into the nascent polypeptides, followed by reacting the azide group of AHA by click chemistry with an alkyne-labelled tag. Our analysis of AHA-tagged peptides demonstrated that the decreased abundance of, for example, ribosomal proteins in aged animals is not solely due to degradation but also reflects a relative decrease in their synthesis. Together our work provides a proteomic snapshot of aging and evidence that the proteostasis network is altered in aged animals, both in physiological conditions and in response to heat stress.

SYM 48-04

FUNCTIONAL AMYLOID FROM FUNGAL HYDROPHOBINS: STRUCTURE, ASSEMBLY AND APPLICATIONS

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Hydrophobins are small soluble fungal proteins which spontaneously assemble into amphiphilic monolayers at hydrophobic/hydrophilic interfaces and serve a number of biological roles. These include aiding spore dispersal, host attachment and infection. Hydrophobin monolayers reverse surface wettability, making them suitable for increasing the biocompatibility of many hydrophobic materials. There are two classes of hydrophobins. Class I hydrophobin assemblies are functional amyloid fibrils and are extremely robust; dissociation is only possible with certain concentrated acids. In contrast, class II assemblies do not contain amyloid structure and can be dissociated readily in organic solvents and detergents. Hence, the two classes of hydrophobins are suited to different applications. We have used a combination of techniques including mutagenesis, electron and atomic force microscopy, solution and solid-state Nuclear Magnetic Resonance (NMR) spectroscopy and data-driven molecular docking to study the structure and properties of the soluble and assembled forms of these remarkable proteins. Our results have allowed us to build a molecular model of hydrophobin monolayers and the assembly process and explain why class I and class II hydrophobins have different physical properties. In addition, we have demonstrated that surface tension can be used to control hydrophobin assembly. Our work open up opportunities to engineer hydrophobin-based products and these applications and possibilities will also be discussed.
SYM-48-05

DECIPHERING THE “AGGREGATION KINETICS” OF MISFOLDED PROTEINS IN THE CELL

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Misfolded proteins can assemble into visible aggregates in cells as a central pathology to neurodegenerative disorders. However, there are various types of aggregates with distinct properties (such as the IPOD and JUNQ) which may arise by distinct engagement strategies of the misfolded proteins with quality control machinery. How and why do these structures form? To get at this question we have built a toolkit to decipher in molecular detail the assembly process of misfolded proteins and relationship to the appearance of different visible aggregate structures (1, 2). Using three misfolded protein systems, polyalanine, polyglutamine and superoxide dismutase we found (polyalanine (37A) and superoxide dismutase 1 (SOD1) mutants A4V and G85R) accumulated into the same JUNQ-like inclusion whereas the other, polyglutamine (72Q), formed spatially distinct IPOD-like inclusions. Sedimentation velocity analysis revealed the SOD1 mutants and 37A have abruptly and constitutively altered oligomeric states with respect to the non-aggregating forms, regardless of whether cells had inclusions or not, whereas 72Q was primarily monomeric until inclusions formed. We propose mutations leading to JUNQ inclusions induce a constitutively “misfolded” state that attract and ultimately overextend protein quality capacity, which leads to aggregation into JUNQ inclusions. PolYQ is not “misfolded” in the classic sense, but prone to stable fibril formation that leads to a distinct clustering pattern in cells and different engagement patterns with cellular machinery. (1) Ramdzan et al (2012) Nat. Methods 9, 467-470; (2) Polling et al (2014) J. Biol. Chem. 289, 6669-6680.

SYM-49-01

PIRNAS IN THE ADULT TESTES IN THE COMMON MARMOSET

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Small RNAs mediate gene silencing by binding Argonaute/Piwi proteins to regulate target RNAs. Here we describe small RNA profiling of the adult testes of Callithrix jacchus, the common marmoset. The most abundant class of small RNAs in the adult testes was priRNAs, while 353 novel miRNAs but few endo-siRNAs were also identified. MARWI, a marmoset homolog of mouse MIWI and a very abundant PIWI in adult testes, associates with the majority of piRNAs that show characteristics of mouse pachytene piRNAs. As in other mammals, most marmoset priRNAs are derived from conserved clustered regions in the genome. However, unlike in mice, marmoset piRNA clusters are also found on the X chromosome, suggesting escape from meiotic sex chromosome inactivation by the X-linked clusters. Some of marmoset piRNA clusters contain antisense-orientated pseudogenes, suggesting regulation of parental functional protein-coding genes. In addition, more piRNAs map to transposable element (TE) subfamilies when they have copies in piRNA clusters. The strand-bias observed for piRNAs mapped to each TE subfamily also correlates with the polarity of copies inserted in clusters. These findings suggest that marmoset piRNA clusters determine the abundance and strand-bias of TE-derived piRNAs, regulate protein-coding genes via pseudogene-derived piRNAs, and may even play roles in meiosis in the adult testis.

SYM-49-02

MICRORNA REPLACEMENT THERAPY IN CANCER – ARE WE THERE YET?

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microRNAs (miRNAs) play critical roles in regulating gene expression for key cellular processes in normal and abnormal physiology. microRNA-7 (miR-7) is a 23 nucleotide miRNA with expression predominantly limited to the brain, spleen and pancreas. miR-7 expression is tightly regulated at the transcriptional and processing levels and it is also the target of circular RNA (circRNA-7). Loss of expression of miR-7 has been linked to the development of accelerated growth, cancer (e.g. head and neck, liver and glioblastoma) and metastasis. As a tumor suppressor, we propose mutations leading to JUNQ inclusions induce a constitutively “misfolded” state that attract and ultimately overextend protein quality capacity, which leads to aggregation into JUNQ inclusions. PolYQ is not “misfolded” in the classic sense, but prone to stable fibril formation that leads to a distinct clustering pattern in cells and different engagement patterns with cellular machinery. (1) Ramdzan et al (2012) Nat. Methods 9, 467-470; (2) Polling et al (2014) J. Biol. Chem. 289, 6669-6680.

SYM-49-03

A MICRORNA-QUAKING REGULATORY PATHWAY CONTROLS ALTERNATIVE SPlicing ASSOCIATED WITH EPITHELIAL CELL PLASTICITY

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Epithelial cell plasticity is fundamentally required for cell differentiation during embryogenesis and contributes to metastatic cancer progression. Previously, we have discovered a family of microRNAs (miR-200 family) that are critical regulators of epithelial-mesenchymal associated plasticity associated with invasive and stem-like properties (1,2). Sequencing of the transcriptome revealed that miR-200 causes widespread changes in alternative splicing, many of which are associated with reversal of epithelial-mesenchymal transition (EMT). By examining clinical and experimental datasets we identified the RNA binding protein, Quaking, as an important miR-200 target. Although Quaking has been implicated in alternative splicing its role in this process has not been well defined. Here, we show that Quaking regulates a large subset of alternative splicing events which are altered by miR-200 during EMT. By examining clinical and experimental datasets we identified the RNA binding protein, Quaking, as an important miR-200 target. Although Quaking has been implicated in alternative splicing its role in this process has not been well defined. Here, we show that Quaking regulates a large subset of alternative splicing events which are altered by miR-200 during EMT. In addition, reduction of Quaking expression phenocopies miR-200 function in attenuating cell migration and invasion. These data indicate that a miR-200-Quaking pathway operates to globally control EMT-associated alternative splicing. Further studies are directed at examining the functions of this pathway and alternatively spliced products in cancer progression. (1) Gregory PA et al (2008) Nature Cell Biol. 10, 593-601. (2) Lim YY, et al (2013) Epigenetic modulation of the miR-200 family is associated with transition to a breast cancer stem-cell-like state. J Cell Sci. 126:2256-66.
DEPOLARISATION-ASSOCIATED MiRNA REDISTRIBUTION IN HUMAN NEURONS OCCURS PRIMARILY IN THE NEURITE FRACTION AND INVOLVES THE RELEASE OF EXOSOMES

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MicroRNA-mediated translational regulation is emerging as a key mechanism underlying synaptic plasticity as these small, non-coding RNAs can provide the exquisite tempo-spatial control required to support this highly specialised functionality. We previously demonstrated dysregulation of miRNA biogenesis and expression in post-mortem tissue in schizophrenia, and thus sought to understand the activity-related dynamics of miRNA expression in living human neuronal cells. We exposed differentiated SH-SYSY cultures to one or four successive K+ depolarisations and profiled miRNA expression changes in isolated neurites and cell bodies by microarray. The neurite fraction was uniquely characterised by a specific pattern of miRNA depletion after activity. Many depleted transcripts were found in exosomes released during depolarisation; unexpectedly, 48% (by relative abundance) of exosomal miRNAs were specific to humans or higher primates. miR-658, the most abundant exosomal miRNA (13% of total miRNA), has only 249 conserved targets, but 1290 non-conserved targets included. Comparative functional analyses found conserved targets performed more basic roles in gene expression and cellular organisation, with no significant neuronal pathways. In contrast, non-conserved targets demonstrated significant contribution to several neuronal signalling pathways. These findings support a role for recently evolved miRNA-miRNA interactions in higher brain functions, and highlight the importance of inclusivity over conservation in the context of neuronal miRNA function.

EXPLORE THE ROLE OF RNA CYTOSINE METHYLATION IN POST-TRANSCRIPTIONAL GENE REGULATION AND CANCER

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5-methylcytosine (mC) in eukaryotic RNA has been known to exist for decades, however, laborious detection methods have limited the understanding of its role. With the availability of high-throughput sequencing techniques, these drawbacks have been overcome, revealing non-random distribution of internal methylation in a wide variety of RNA biotypes. Recently, we implemented a bisulphite sequencing-based technique for transcriptome-wide (bsRNA-seq) detection of mC© and mapped thousands of mC© sites in the human transcriptome including in mRNA and non-coding RNA. Biased distribution of mC© within mRNAs e.g. enrichment in the untranslated regions, is consistent with a role in post-transcriptional gene regulation. We have investigated the enzymes responsible for modifying RNA by coupling RNAi-mediated knockdown with bsRNA-seq, identifying hundreds of candidate mC© sites that are targeted by the two RNA methyltransferases, NSUN2 and TRDMT1. Interestingly, both RNA methyltransferases have a link to cancer; TRDMT1 is inhibited by the anti-cancer drug, 5-azacytidine, and NSUN2 is overexpressed in tumours. As such, we are investigating the role of RNA methylation in cancer by comparing the mC© profiles of normal prostate cells (PrEC) and metastatic prostate cancer cells (LNCaP). Analysis of the recorded patterns of mC© sites in RNA shows many transcripts are differentially methylated between each cell line. We are currently consolidating and extending the potential link of mC© to post-transcriptional gene regulation and cancer, as well as addressing its molecular function.
SYM-50-03
REVEALING HOW THE FIRST TISSUE-LIKE STRUCTURE FORMS IN THE MAMMALIAN EMBRYO

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We have recently designed new imaging tools to study how mammalian cells interact with neighbouring cells during the earliest stages of embryonic development. Using in vivo imaging of developing mouse embryos, we have identified a new role for Cadherin-dependent filopodia in controlling how cells change their shape and position to form the first tissue-like structure. Our findings reveal some of the dynamic mechanisms controlling key cell functions in living mammalian organisms.

SYM-50-04
RHO GTPASE FUNCTIONS IN EARLY POSTIMPLANTATION MOUSE DEVELOPMENT

Loebel D.A.F.1,2, Tang T.L.1, Chung H.C.1, Power M.2, Greenlees R.2 and Tam P.P.L.1,2
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Members of the family of Rho-related small GTPase proteins play multiple roles in cellular function and embryonic development, including establishment and maintenance of cell polarity, organization of the actin cytoskeleton, orientation of cell division, cell junction formation and cell migration. One RhoGTPase, Cdc42, is expressed broadly in the embryo, and loss of Cdc42 leads to demise of embryos shortly after implantation. To investigate the role of Cdc42 later in germ layer formation and early organogenesis, a strategy of timed deletions of Cdc42 was employed using the tamoxifen inducible Cre, Ubc-CreERT2. After tamoxifen administration at E5.5-6.5, embryos contained tissues that correspond to the three primary germ layers, but did not form a foregut pocket or somites and the neuroectoderm did not form a proper polarized pseudostratified epithelial layer. Tamoxifen injection at E7.5-8.5, or endoderm-specific deletion mediated by Foxa2-merCremer, resulted in the formation of smaller endoderm-derived organs, consistent with a role for Cdc42 in early stages of organ development. Knockdown of Cdc42p63, encoding a Cdc42-interacting protein with highly restricted expression in the pharyngeal endoderm affects apico-basal polarity of cells and lumen formation in cystic structures in vitro. These findings reveal new roles for Cdc42 and its interacting proteins in epithelial morphogenesis during early postimplantation development.

SYM-50-05
INTRAVITAL IMAGING AT SUBCELLULAR RESOLUTION REVEALS MULTIPLE ACTIN FILAMENT POPULATIONS INVOLVED IN EXOCYTOSIS OF SECRETORY GRANULES

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We established an intravital microscopy-based system to study actin dynamics in secretory granules (SGs) during exocytosis in salivary glands of living mice and rats. Using transgenic mouse models that express selected fluorescently labeled molecules, we discovered several key aspects of exocytosis that were not seen in ex vivo models. In the current study we sought to explore how tropomyosin (Tms), which form co-polymers along the length of actin filaments, may be modulating the actomyosin scaffold, and hence controlling exocytosis. There are about 40 different Tm isoforms and they are known to regulate the actin cytoskeleton by specifying the functional characteristics of actin filaments in time and space either via either a direct effect on filament stability or by regulating recruitment of actin binding proteins and myosin motors. We found that the Tm4 and Tm5NM1 isoforms line the apical canaliculi of the acinar structures. Upon stimulation with isoproteranol Tm5NM1 and Tm4 were recruited onto the fused SGs together with F-actin, but with different kinetics to that of bulk filaments. Intravital microscopy of transfected rat salivary glands revealed that Tm5NM1 and Tm4 were recruited after fusion of the SGs with the apical plasma membrane and coincided with F-actin recruitment to the granules. Genetic ablation of Tm5NM1 or exposure to an anti-Tm compound altered the kinetics of granule exocytosis but did not prevent the completion of granule exocytosis. We conclude that Tm isoforms are present at the apical membranes and, in the case of Tm5NM1 and Tm4, directly participate in actin scaffolding and gradual delivery of granule contents post-fusion.

SYM-51-01
CHARACTERISING NOVEL SIGNALING AXES IN THE INTERFERON SYSTEM

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The type I interferons (IFNs) are a critical multigene family with key roles in the immune response. There is evidence that different IFN subtypes such as IFN α's and IFNβ are produced by different cells in response to different stimuli consistent with them having different regulatory mechanisms. However there has been no molecular data to support different mechanisms of action. Type I IFNs typically interact with cell surface receptors IFNAR1 (low affinity) and IFNAR2 (high affinity); then activating JAK-STAT signal transduction pathways. We describe a novel interaction that can occur for IFNβ, not IFNα, with IFNAR1; and using X-ray crystallography have solved a structure that provides a detailed mechanism for this selectivity. Furthermore we demonstrate that in the absence of IFNAR2 on cells, the IFNβ:IFNAR1 complex can internalise and activate a unique set of genes, although it does not activate the conventional JAK-STAT signalling pathways and associated genes. IN vivo, this unique IFNβ:IFNAR1 signaling axis plays an important role in the pro-inflammatory properties of IFNβ in models of disease such as septic shock. This new signaling axis provides new avenues for therapeutic and diagnostic advances in regulating inflammation.
SYM-51-02

ID4 CONTROLS MAMMARY STEM CELLS AND MARKS BREAST CANCERS WITH A STEM CELL-LIKE PHENOTYPE

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Basal-like breast cancer (BLBC) is a heterogeneous poor prognosis disease, however its cellular origins and etiology are poorly understood. In this study, we show that ID4 is a key regulator of mammary stem cell self-renewal and marks a subset of BLBC with a putative mammary basal cell of origin. Using a novel ID4-GFP knock-in reporter mouse and single cell transcriptomics, we show that ID4 marks a stem cell-enriched subset of the mammary basal cell population. ID4 maintains the mammary stem cell pool by suppressing Notch, Brcar1 and Elf5, key factors required for luminal differentiation. Furthermore, ID4 is specifically expressed by a subset of BLBC that possess a very poor prognosis and a transcriptional signature similar to a mammary stem cell. These studies identify a new mammary stem cell regulator, de novo the heterogeneity of BLBC and link a subset of mammary stem cells to the etiology of BLBC.

SYM-51-03

PATHWAY AND NETWORK ANALYSIS OF MOLECULAR INTERACTIONS AND SIGNALLING NETWORKS IMPLICATED IN EPITHELIAL-MESENCHYMAL PLASTICITY

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Epithelial mesenchymal transition (EMT) is the process whereby polarized epithelial cells alter the expression of key adhesion and regulatory molecules, and gain the ability to survive and migrate as single cells. While EMT is a normal developmental process, we now recognize that processes involved in cancer metastasis have many elements in common with this transition, including single cell dispersal, increased migratory and invasive potential, and common changes in gene expression, suggesting that cancer cells subvert this normal cellular transition to allow metastatic spread. Pathway analysis is a popular computational technique for dissecting the role of the cells many signal transduction pathways in regulating phenotypic shifts such as that seen in EMT. In this presentation, I will demonstrate how knowledge-based approaches to network analysis are able to integrate heterogeneous experimental data and describe the use of pathway analysis techniques to identify candidate signalling networks implicated in EMT in cell line models of breast cancer metastasis.

SYM-51-04

BETAL-ADRENERGIC SIGNALLING REGULATES INVADOPODIA FORMATION AND INVASION IN BREAST CANCER

Creed S.1, Hassan M.1, Chan K.T.1, Albold S.1, Berginski M.E.1, Bear J.E.1,2, Nowell C.J.1 and Sloan E.K.1
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To overcome physical boundaries imposed by the tumour microenvironement, many invasive cancer cells form specialized protein complexes called invadopodia which actively degrade the surrounding matrix. β-adrenergic receptor (βAR) signalling has been shown to affect tumour cell invasion, however, the cellular basis by which this occurs is still not well understood. To address this, we investigated effects of βAR activation on the formation of invadopodia in breast cancer cells. βAR increased both the percentage of invadopodia positive cells and number of invadopodia per cell in MDA-MB-231 cells. Investigation using selective ligands demonstrated β2AR - but not β1AR - signalling is required for increased invadopodia. Ectopic expression of β2AR induced invadopodia formation in MCF7 cells, which endogenously express predominately β1AR and otherwise do not form invadopodia. Consistent with a role for invadopodia in tumour cell invasion, β2AR activation enhanced 3D migration of breast cancer cells both in vitro and from primary tumour explants. Pre-treatment with the Src inhibitor PP2 blocked both invadopodia formation and 3D migration following β2AR activation, showing that β2AR invadopodia formation is dependent on Src. Our results identify β2AR as a novel molecular regulator of invadopodia formation, and highlight a mechanism for the effects of stress responsive signalling on progression of cancer metastasis.

SYM-51-05

THE ACTIN ASSOCIATED PROTEIN TROPOMYSOSIN 5NM1 PARTICIPATES IN MAPK/ERK-MEDIATED CELL PROLIFERATION BY REGULATING PERK NUCLEAR TRANSPORT

Wang B.1, Schevzov G.1, Hook J.1, Kee A.1, Lucas C.1, Seger R.1, Hardeman E.1, and Gunning P.1
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An array of structurally and functionally distinct populations of actin filaments co-exists within cells. Actin filaments are known to play a critical role in mediating signalling pathways involved in cell proliferation, although the specific molecular mechanism(s) still remain unclear. We have previously shown that the family of actin associated proteins tropomysosin (Tm) defines the function of specific actin filament populations in an isofrom-specific manner. In this study, we demonstrate that a specific population of actin filaments containing the Tm5NM1 isofrom can regulate cell proliferation using primary mouse embryonic fibroblasts (MEFs) from wild type (WT), and Tm5NM1 overexpressing transgenic (Tg) and knockout (KO) mice. Tm5NM1 KO MEFs display a significant reduction in the cell proliferation rate; whereas, an increase in proliferation rate is evident in Tg MEFs. Antibody microarray analysis revealed a dysregulation of the MAPK pathway in the KO MEFs. Studies comparing specific MAPK signalling pathway inhibitors showed that MEF proliferation is insensitive to ERK2 inhibition in the absence of Tm5NM1. The activation and nuclear entry of phosphorylated ERK1/2 requires dual phosphorylation on two distinct motifs, initially by MEK followed by casein kinase 2 (CK2). We observed that Tm5NM1 is required for CK2-mediated pERK nuclear translocastion as the inhibition of CK2 reduced the proliferation of WT MEFs, but not KO MEFs. Proximity ligand assays further demonstrated that the interaction of pERK with its nuclear transport partner importin-7 is impaired in KO MEFs. Taken together our data indicates that Tm5NM1 participates in MAPK/ERK mediated cell proliferation by regulating the nuclear transport of pERK.
SYM-52-01
INTRODUCTION TO THE LIFE AND WORK OF WARWICK HILLIER
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SYM-52-02
STUDIES OF PHOTOSYSTEM II AT THE HILLIER LAB: MECHANISM OF PSI PHOTODAMAGE & MECHANISM OF WATER OXIDATION BY PSI
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Ongoing studies of Photosystem II (PSII) at the Hillier Lab are focused on two areas: (1) mechanism of photodamage of PSII and (2) mechanism of water oxidation investigated using $^{18}$O isotopic fractionation. (1) The current hypotheses of PSII photodamage are due to either (i) donor/acceptor side limitations due to excessive light energy or (ii) direct photo-inactivation of the Mn$_4$CaO$_5$ cluster. Our results show that across a large range of light intensities, there is a fast and slow phase in the rate of photodamage of PSII in vitro. Furthermore, the rate of photodamage is not linearly dependent on light intensity. The possibility of photodamage occurring via parallel pathways is presented based on available data obtained at the Hillier Lab. (2) Kinetic isotope effect (KIE) is a powerful tool in examining reaction mechanism as KIE reflect changes in bond organisation during the course of reactions. Examination of $^{18}$O KIE during water oxidation by the Mn$_4$CaO$_5$ in PSII is therefore a critical piece of the puzzle in our efforts to gain greater understanding of this reaction mechanism. Development of methods to measure $^{18}$O KIE using membrane inlet mass spectrometry at the Hillier Lab will be presented.

SYM-52-03
MONITORING PHOTOSYSTEM II FUNCTION IN LEAVES
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Given its unique function of light-induced water oxidation and its inevitable susceptibility to photoinactivation normally during photosynthesis, Photosystem II (PS II) is often the focus of studies of photosynthetic structure and function, particularly in environmental stress conditions. There are a number of approaches for quantifying or monitoring PS II functionality or the stoichiometry of the two photosystems in leaf segments. (1) Chlorophyll fluorescence parameters are convenient to derive, but the information-rich signal suffers from the localized nature of its detection in leaf tissue. (2) The gross oxygen yield per single-turnover, saturating flash in CO2-enriched air gives a more direct, quantitative measure of the functional PS II content, assuming that each functional PS II evolves one oxygen molecule after four flashes. However, the gross oxygen yield per single-turnover flash (multiplied by four) could over-estimate the content of functional PS II if mitochondrial respiration were lower in flash illumination than in darkness. (3) The cumulative delivery of electrons from PS II to P700+ (oxidized primary donor in PS I) after a single-turnover flash is added to steady background far-red light is a relative, whole-tissue measure of functional PS II, and can be used to track the recovery of PS II from photoinactivation. It can further yield the established stoichiometry of the two photosystems if one assumes that the rate of photo-oxidation of P700 per se is a second-order reaction. Chow et al. (2012) Photosynthesis Research 113: 63-74; Hu et al. (2013) Photosynthesis Research 117: 517-528; Jia et al. Physiologia Plantarum (2014) doi:10.1111/ppl.12235.

SYM-52-04
HIGH LEVEL COMPUTATIONAL CHEMICAL STUDIES OF SUBSTRATE WATER BINDING TO THE OXYGEN EVOLVING CENTRE (OEC) IN PHOTOSYSTEM II
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Pioneering studies by Hillier, Wdzydzinski and Messinger, using $^{18}$O labeled water, determined the exchange rates of the two substrate water molecules with the oxygen evolving Mn$_4$/Ca site in the OEC. These results are remarkable and counter-intuitive. Two rates are seen, ‘fast’ and ‘slow’ in all OEC catalytic intermediate S states. The fast rate exceeds the slow by ~ 2-4 orders of magnitude, with some evidence of monotonic decline with increasing S state (and Mn oxidation levels), but the slow rate does not. This rate is comparable in the S$_1$, S$_2$ and S$_3$ states, but slows by at least one hundred-fold in S$_5$. The rates are relatively insensitive to a range of biochemical perturbations (pH change, peripheral protein removal, Ca/Sr replacement) and site directed mutational changes, all of which still preserve overall function. The variations are generally < 5 fold, compared to optimal values. These data have totally challenged interpretation within currently favoured views of the OEC manganese cluster oxidation levels and substrate water binding locations. In these ‘high’ Mn oxidation state models (mean values of ~$3.5$ in S$_2$), the substrates are bound as hydroxy or oxy bridge species, which progressively deprotonate as S states advance. Broad chemical experience shows that such oxyl species have exchange rates far slower than seen for the OEC substrates. A new quantum chemical model of the OEC (Pace, Stranger et al. Ang. Chem. 2012, 51,12025) indicates that the Mn oxidation levels in the OEC are lower than previously believed (~$3.0$ in S$_2$) allowing the substrates to bind as waters up to S$_5$. Calculated substrate binding energies are consistent with those seen experimentally by Hillier et al. and this new model, with its implications for the OEC catalytic mechanism, will be described.