SYMPOSIA

Tuesday - Thursday
SYM-01-01

MAKING EPIDERMAL BLADDER CELLS BIGGER: THE ROLE OF ENDOPOLYPLOIDY IN SALINITY TOLERANCE OF A MODEL HALOPHYTE PLANT

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Epidermal bladder cells (EBC) are modified trichomes found on plants in the Aizoaceae and Chenopodaceae, which morphologically resemble small water filled balloons rather than hairs. In Mesembryanthemum crystallinum, these cells swell as the plant ages and largest EBC are found on the abaxial epidermis of leaves, as well as on stems and flower buds of salt-treated plants. Here we report that the rapid expansion of the cells due to endopolyploidy, DNA replication occurs in the absence of mitosis, driven by both developmental and environmental cues. Increasing ploidy levels are observed as leaves expand, and salt-treatment leads to a further increase in endopolyploidy, with ploidy levels up to 6X greater estimated for EBC on the flower buds of salt treated plants compared to untreated plants. Ploidy increases in these cells are accompanied by large increases in the size of the nucleus with diameters of up to 140 microns routinely measured. Mining of EBC transcriptomic data for candidate genes with known roles in cell cycle control, cell size regulation and cytokinetic components identified genes with significant changes in salt-treated plants. It has been proposed that increased ploidy helps to mitigate stress damage, and may contribute to tolerance by increasing the store size for sodium sequestration and facilitating higher cellular metabolic activity. This study shows that M. crystallinum is an outstanding model for studying endopolyploidyization and its physiological role in relationship to both development and environmental stress tolerance. The authors would like to acknowledge financial support from SCPS and an SCU seed grant.

SYM-01-02

A MOLECULAR MECHANISM FOR HOW PLANTS MAINTAIN THEIR CELLULOSE PRODUCING CAPACITY DURING SALT STRESS

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Abiotic stress, such as salinity, drought and cold, causes severe yield losses for most plant crop species. Understanding mechanisms for how to improve plants ability to produce biomass, which largely is constituted by the plant cell wall, during abiotic stress are therefore of utmost importance for agricultural activities. Cellulose is a major component of the cell wall, and is synthesized by microtubule-guided cellulose synthase enzymes at the plasma membrane. We have identified two new components of the cellulose synthase complex, which we call Companions of Cellulose Synthase (CC) proteins. The cytoplasmic tails of these membrane-spanning proteins bind to microtubules and promote their polymerization. This activity supports microtubule dynamics and cellulose synthase localization at the plasma membrane, and renders seedlings less sensitive to salt stress. The seminar will provide molecular insights into how the CC proteins work to reestablish microtubules and cellulose synthesis after salt stress exposure. Hence, our findings offer a mechanistic model for how the CC proteins sustain microtubule organization and cellulose synthase localization, and thus how they aid plant biomass production, during salt stress.

SYM-01-03

REVEALING THE ROLES OF CALCIUM TRANSPORTERS IN RESPONSE TO HYPOXIA AND COMBINED HYPOXIA AND SALINITY STRESS IN ARABIDOPSIS

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1 School of Land and Food, University of Tasmania, Hobart, Tasmania 7001, Australia. 2 School of Science and Health, Hawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW 2751, Australia. 3 School of Plant Biology and Institute of Agriculture, The University of Western Australia, Crawley, WA 6009, Australia. Over 17 million km² of land is affected by flooding, resulting in substantial yield losses and jeopardising food security across the globe. Besides the increasingly severe flooding events, the occurrence of the combined salinity and waterlogging stress is also increasing. The role of Ca²⁺ in response to abiotic stress has been widely recognized in plants but still poorly understood within specific cell types under different root zones under hypoxia stress or combined hypoxia and salinity stress. Whole-plant physiological and tissue-specific Ca²⁺ changes were studied using knock-out Arabidopsis mutants of CAX+/−, AtPASE (ACA), CAX²⁺-proton exchanger (CAX), and respiratory burst oxidase homolog D (RBOHD) in waterlogging treatment or combined stress. In the wild-type (WT) plants, the expressions of ACA8, CAX4, CAX11 and RBOHD were down-regulated by up to 3-fold by hypoxia treatment. CAX2 accumulation in root tissues was much higher in steller cells in the mature zone of aca8, aca11, cax4 and cax11 mutants. In addition, we also show that CAX1 plays a key role in maintaining cytosolic Ca²⁺ homeostasis and/or signalling in root cells under hypoxic conditions. Phenotyping experiments found that waterlogging stress caused most severe damage to both WT and rohod compared to salinity or combined hypoxia or hypoxia and salinity stress. What’s more, robhoD was more sensitive to salinity, waterlogging or combined stress than WT. After pretreated with 48 h of salinity stress, transgenic Arabidopsis seedlings make rbohD absorb more Na⁺ and Cl⁻ from elongation and mature zones than WT. In most tissues except the elongation zone in robhoD, the H2O2 concentration had decreased after 1 h of hypoxia, but then increased significantly after 24 h treatment in elongation and mature zones, further suggesting that RBOHD may shape hypoxia-specific Ca²⁺ signatures via the modulation of apoplastic H2O2 production. In summary, CAX2-efflux systems especially CAX11 and RBOHD play critical roles in plant adaptive to hypoxia stress by shaping the stress-specific Ca²⁺ signatures. As dependence and reliance of mammalian system on O2 is much stronger than in their plant counterparts, acute responses to hypoxia must operate within a timeframe of seconds. O2-regulated ion channels fit this description very well. To further understand the mechanisms by which plants sense low-oxygen stress, we first summarise and identify several known candidates for oxygen sensing in the mammalian literature. We then identify key oxygen sensing domains (PAS, GCC, GAF, PHD) in mammalian systems and use the sequences of those oxygen sensing domains to identify the potential plant counterparts in Arabidopsis. Several plasma- and tonoplast based Ion channels (such as TPC1) with predicted oxygen sensing ability were identified in plants.

SYM-01-04

CO-EXPRESSION MODULES LINK AQUaporin GENE EXPRESSION TO PHYSIOLOGICAL PARAMETERS DURING DROUGHT-REHYDRATION IN ARABIDOPSIS THALIANA

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The ability of plants to survive stresses like drought determines their success and ultimately yield in farming systems. Aquaporins are molecular channels in plant membranes that provide a gating mechanism for water fluxes and other small molecules. They are encoded by a family of 35 genes in Arabidopsis thaliana, which show characteristic changes in expression in response to environmental stresses. Previous research has found connections between aquaporins and drought tolerance. This study aims to investigate how changes in physiological parameters during drought-rehydration events are related to changes in aquaporin expression. Drought-rehydration and abscisic acid (ABA) watering experiments were conducted, using two different well-grown Arabidopsis thaliana plants. ABA watering was used to mimic the stomatal response to drought, without the effect of water deficit. Physiological parameters, like soil water potential, stomatal conductance, relative water content, and leaf ABA, were measured and related to gene expression in the leaves. Aquaporins were clustered into distinct groups according to their expression pattern. A predominant cluster of down-regulated genes showed a good linear correlation with stomatal conductance, while a cluster of up-regulated genes showed a response similar to genes involved in the ABA signaling pathway. However, no direct regulation by leaf ABA was observed. Compared to drought, ABA watering produced a different pattern of changes in gene expression. These results indicate that aquaporin gene expression may be regulated in different networks during environmental stress adaption and potentially fulfill functions in hydraulic and ABA signal transduction.
SYM-01-05
AN -OMICS APPROACH TO IDENTIFY THE REGULATORS OF VEGETATIVE DESICCATION TOLERANCE IN RESURRECTION PLANTS

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While the majority of angiosperms produce desiccation tolerant seeds, vegetative desiccation tolerance (VDT) is a rare trait, reported from 140 species collectively known as resurrection plants. As genes that display seed-specific expression in other plants (e.g. seed storage proteins) are activated in resurrection plants during dehydration, it is thought that VDT evolved through recruitment of the LAFL regulatory network that controls seed maturation. However, there is no experimental evidence to support this hypothesis. Using next-generation sequencing, we assembled the Xerophyta humilis genome, and analysed the transcriptome in shoots during dehydration, and in seeds during maturation. Orthologs of the LAFL transcription factors (TFs) including LEC1, LEC2, and ABI3 were detected in the X. humilis genome, but their expression profiles during seed maturation and vegetative desiccation differed markedly. While all were expressed in seeds, with LEC1 strongly up-regulated during maturation, only ABI3 was expressed in shoots, but at very low levels with no evidence of up-regulation during dehydration. That ABI3 transcripts detected in shoots lack the B3 binding domain, and the RY-element recognised by ABI3 is not conserved in the promoters of X. humilis orthologs of known ABI3 targets in Arabidopsis, indicates that the up-regulation of these genes observed during dehydration is not driven by ABI3. Instead, we found enrichment of the abscisic acid response element in this gene-set and also observed up-regulation of ABF TFs, which are activated by drought in vegetative tissues of other plants. VDT in X. humilis is not associated with re-activation of seed master regulators, but may instead involve activation of seed-genes by vegetative drought response regulators.

SYM-02-02
IS THERE A ROUTE TO MARKET FOR A GENETICALLY-MODIFIED BUT HEALTH-PROTECTING PRODUCT?

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Back in 2008 we published a paper in Nature Biotechnology on an anthocyanin-enriched purple tomato which, when fed to cancer-prone mice, extended their life span by 30%. This work received global dissemination and was very well received, and yet our purple tomatoes are still not available to consumers. Why not? How can a small, academically-oriented company get an evidence-based product to those that would like to consume it? I will describe problems associated with a traditional patent and licence approach. I will describe problems associated with re-activation of seed master regulators, but may instead involve activation of seed-genes by vegetative drought response regulators.

SYM-02-01
COMMERCIALISING OMEGA-3 EGGS: PRODUCTION AND BIOAVAILABILITY

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Dietary omega-3 long chain polyunsaturated fatty acids (n-3 LCPUFA, such as eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA) are essential for normal development and healthy living. However, most Australians do not meet the recommended daily intake of n-3 LCPUFA and so many people take omega-3 supplements in the form of fish or krill oil. However, there are issues about the sustainability of further increasing pressure on already fully exploited marine resources. Chicken eggs are already a source of long chain omega-3 but it is known that we can further increase the omega-3 content by feeding the chickens either fish oil, or a sustainable plant oil (such as flaxseed oil) which is high in the short chain omega-3 fatty acid-alpha-linolenic acid (ALA) and under the right dietary circumstances the chicken can convert some of this ALA into the ‘fish-type’ EPA and DHA. Over several years we refined this approach such that recently we could trial it in a semi-commercial setting. This results of the trial showed: increases in egg omega-3 content (6-fold more omega-3. 2.5-fold more long chain omega-3), no detrimental effect on egg production (>90% eggs/day), and no significant effect on egg sensory attributes and acceptability. The acute effects of eating 2 omega-3 eggs on blood fatty acid profiles in human consumers showed significant increases in the omega-3 content of triglyceride and phospholipid fractions for at least 4 hours. These results are an important step towards delivering omega-3 enriched eggs to consumers by feeding chickens with a sustainable plant oil.

SYM-02-03
DEVELOPMENT AND USE OF AN ULTRA-LOW GLUTEN BARLEY

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Gluten proteins in cereals are a serious health challenge for people with coeliac disease and non-coeliac gluten sensitivity. Coeliac disease occurs in about 1% of the population, and requires lifelong exclusion from the diet of the various gluten proteins found in wheat (gliadin and glutenins), barley (hordeins), rye (secalins), and for some oats (avenins). Untreated coeliac sufferers suffer from painful intestinal malignancy, and greatly damaged mucosal villi, resulting in poor absorption of nutrients. They frequently suffer from low bone density, and their alternative diets are often low in fibre and high in fat and sugar. To address these issues we identified and combined null barley mutations of each of three classes of hordein. The triple-null barley lines, called Kebari, have more than 10,000 fold reduction in gluten compared to control barley (1). Other off-target changes to grain composition will be reported. The initial hulled Kebari lines had smaller grains, making processing and malting less efficient. With further breeding the seed weight and agronomic performance has been largely recovered. We have also developed a hull-less version of Kebari suitable for use in the food industry, in order to make healthy cereal options for gluten avoiders. The hordein levels in these Kebari lines are well below the WHO recommended level of 20 ppm for classification as gluten free (1). Kebari is already being used in Germany to make a gluten free barley beer, and it is anticipated a growing number of food and beverage options will soon become available. (1) G. J. Tanner, M. J. Blundell, M. L. Colgrave, and C. A. Howitt. Creation of the first ultra-low gluten barley (Hordeum vulgare L.) for coeliac and gluten-intolerant populations. Plant Biotechnology Journal, 14: 1139-1150, 2016.
SYM-02-04

THE USE OF ASSOCIATION MAPPING TO IDENTIFY GENES CONTROLLING PRE- AND POST-FERTILISATION REPRODUCTIVE TRAITS IN BARLEY

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Barley is a diploid cereal crop used in the feed and brewing industries, as well as being a niche functional food for humans due to its high nutritional qualities. The benefits of the barley grain are derived mainly from the endosperm, which along with the embryo, is produced after fertilisation of the embryo sac within the ovule. During early stages of seed development, nutrients are released from maternal ovule tissues and transferred into the endosperm, which concurrently differentiates on a radial axis to form two prominent cell types, the peripheral aleurone and the inner starchy endosperm. We have been studying early grain development in barley with a view to understanding maternal contributions to grain size, weight and morphology, in addition to cues that influence endosperm differentiation. We have developed several microscopic assays to quantify sub-apical details of ovule and grain development, and applied these in a panel of 165 spring 2-row barley cultivars. Association mapping identified multiple genomic regions that contribute to phenotypic variation in these reproductive traits. To identify candidate genes contributing to the phenotypic variation we have used a combination of RNAseq profiling, exome capture and plant transformation. The fundamental knowledge generated in this project is providing novel insight into how different tissues and cells contribute to grain development. This knowledge may be applied in future to tailor specific reproductive traits for improvements in grain yield and composition.

SYM-02-05

GRAIN NITROGEN FILLING IN TWO CONTRASTING AUSTRALIAN WHEAT CULTIVARS

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Wheat (Triticum aestivum L.) is the world’s most widely adapted crop and Australia’s most important crop export. Though yields are generally low compared to other wheat-producing areas, Australian wheat is often superior in quality. Grain quality (protein content) often displays an inverse relationship to yield, where average rainfalls can enhance protein levels and excess rain results in a decrease in grain quality. Grain nitrogen (N) content is dependent on the ability of plants to remobilize N from vegetative tissues and to transport it to the grain. To study grain filling, we selected two Australian prime hard wheat cultivars, Spitfire and Gregory that show contrasting relationships between protein vs yield content. In general, both lines produce similar yields, but Spitfire consistently produces higher grain protein content (GPC). To understand the mechanism controlling GPC, we characterised both lines using both field and controlled growth environments. In both studies, Gregory displayed longer flag leaves, heads and stems but a reduced number of tillers compared to Spitfire. Gregory seeds were smaller but had comparable weights to Spitfire seeds. Study of yields and GPC confirmed similar yields between cultivars but Spitfire presented higher GPC than Gregory. A metabolomic analysis of the seed content during grain filling has been initiated and preliminary results suggest altered amino acid loading into developing seeds occurs between the two lines. In summary, our results show contrasting growth characteristics between Gregory and Spitfire. Further experiments will characterise the mechanisms controlling Spitfire and Gregory grain N filling using both metabolic and transcriptional-based analysis.

SYM-03-01

MOLECULAR MECHANISMS REGULATING GERMLINE STEM CELL REGENERATIVE POTENTIAL AND FUNCTION

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Sustained spermatogenesis in adult males and recovery of fertility following germ cell depletion are dependent on undifferentiated spermatagonia with self-renewal potential. We have previously demonstrated a critical role for the transcription factor spalt-like 4 (SALL4) in spermatogonial differentiation. However, it remains unclear whether SALL4 has broader roles within the spermatogonial pool of proepicardial cells to the heart and expansion of the epicardium. the transcription factor Wt1 has been described as essential for the formation of proepicardial villi, and subsequent transfer of some epicardial cells to the heart and expansion of the epicardium. However, Wt1 mutants are reduced, discontinuous in places, with poor epithelial identity, and a proportionally excessive content in mesenchymal-like cells. This data shows that Wt1 has a much earlier role in epicardial development than SALL4 in maintenance of undifferentiated spermatogonial activity and identifies new cellular pathways regulating the regenerative response of germline stem cells.

SYM-03-02

WT1 IS REQUIRED FOR PROEPICARDIAL DEVELOPMENT

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The epicardium (most external cell layer of the heart) has a dynamic and morphologically complex development. We identified marker combinations that enabled detection and quantification of epicardial progenitors by flow cytometry. This methodology permitted accurate and sensitive analysis of (1) the emergence of epicardial progenitors within the proepicardium (2) their transfer to the heart and expansion as epicardium, and (3) the subsequent epithelial-to-mesenchymal transition (EMT) of some epicardial cells to create the subepicardium mesenchyme. The transcription factor WT1 has been described as essential for epicardial EMT. Using our quantitative methodology, we found that WT1 has a much earlier role in epicardial development: WT1 is required for the formation of proepicardial villi, and subsequent transfer of proepicardial cells to the heart. At later stages, the epicardium of WT1 mutants is reduced, discontinuous in places, with poor epithelial identity, and a proportionally excessive content in mesenchymal-like cells. This data shows that WT1 has a much earlier role in epicardial development than suspected, in epicardial formation and maintenance.
**SYM-03-03**

**PROMOTING MYELIN ADDITION TO THE MATURE BRAIN**

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Oligodendrocyte progenitor cells (OPCs) have the capacity to detect and respond to changes in neuronal activity. In this way, experimentally increasing neuronal activity rapidly increases oligodendrogenesis and myelination in the adult brain. We aimed to determine whether repetitive transcranial magnetic stimulation (rTMS), a non-invasive form of brain stimulation, could similarly influence OPCs. P90 Pdgfr-CreERT2+::Rosa26-YFP and Pdgfr-CreERT2+::TaumGFP transgenic mice were used to fluorescently label OPCs and the oligodendrocytes they produce. We used a 120mT mouse rTMS coil to deliver a sham stimulation or 600 pulses of rTMS as a 10Hz, intermittent theta burst (iTBS) or continuous theta burst pattern to mice. 14 days of iTBS treatment significantly increased the number of new oligodendrocytes detected in the motor and visual cortex and increased the length of myelin internodes laid down. 10Hz and continuous theta burst stimulation did not influence oligodendrogenesis. These data indicate that rTMS can be used to regulate OPC behaviour and promote myelin addition to the mature brain, making it an appealing option for the treatment of remyelination.

**SYM-03-04**

**YAP REGULATES ANABOLIC GLUCOSE METABOLISM TO ENABLE OPTIMAL ORGAN GROWTH**

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The Hippo pathway and its nuclear effector Yap play a central role in the regulation of organ size and cancer. While many modulators of Hippo activity have been identified, little is known about how Yap target genes responsible for the remarkable effects on tissue growth. Here, we show that defects in hepatic progenitor potential and liver growth in Yap-/- and Prox1 mutation zebrafish are caused by impaired glucose transport and nucleotide biosynthesis. Transcriptomic and metabolic profiling revealed that Yap directly regulates expression of glucose transporter glu1, causing decreased glycolytic flux into anaobolic nucleotide biosynthesis in yap-/- and impaired glucose tolerance in adults. We find that nucleotide supplementation improved Yap-deficient phenotypes, indicating the functional importance of glucose-fuelled nucleotide biosynthesis. Furthermore, we demonstrate that the regulation of glu1 and glucose uptake by Yap is conserved in mammals. Our results identify Glu1 as a direct Yap target influencing glucose uptake and utilization for anaoblic nucleotide biosynthesis, which are required for organ growth. Our findings demonstrate the central role of Hippo signalling in metabolic homeostasis.

**SYM-03-05**

**DEFINING THE MECHANISMS BY WHICH GATA2 PROGRAMS LYMPHATIC VESSEL VALVE DEVELOPMENT**

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We and others recently demonstrated that heterozygous germline mutations in GATA2 underlie Emberger syndrome, a disorder characterised by lymphedema and predisposition to myelodysplastic syndrome (MDS)/acute myeloid leukaemia (AML). While GATA2 has well established roles in haematopoiesis, nothing was known regarding the roles of GATA2 in the lymphatic vasculature. Our work has revealed that GATA2 is present at high levels in lymphovenous and lymphatic vessel valves and is required both for the initiation of valve development and for the maintenance of valve architecture. To investigate the transcriptional mechanisms by which GATA2 orchestrates lymphatic vessel valve development, we performed GATA2-ChIP analysis and identified a potential enhancer element 1kb upstream of the Prox1 gene. ChIP-seq data generated using transcription factors critical for valve development (PROX1, FOXC2, NFTAC1 and GATA2) show a cooperative binding pattern at the PROX1 -11kb region. Using transgenic mice we demonstrated this enhancer element has the capacity to drive reporter gene expression in the lymphatic vasculature and at particularly high levels, in valves. CRISPR mediated deletion of the enhancer results in approximately 40 percent of Prox1 -11kb +/Δ animals dying at or shortly after birth. In addition, Prox1 -11kb +/Δ and +/+ mice exhibit phenotypes associated with valve dysfunction including oedema and blood filled lymphatic vessels. Current work aims to define the transcriptional mechanisms by which the Prox1 -11kb enhancer element controls valve development.

**SYM-04-01**

**STYRENE MALEIC ACID: AN AMPHIPATHIC POLYMER TO SOLUBILISE AND STABILISE MEMBRANE PROTEINS**

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To the biochemist, membrane proteins promise fascination and frustration in equal measure. Though crucial to life and highly relevant to many diseases, proteins that have evolved to exist within the complex chemistry of the lipid bilayer rarely prosper when removed from it. Through years of intricate method and process development we are now able to isolate many of them from a range of cell types, and study their structures and functions when isolated in conventional head- and tail-detergents. Unfortunately, in some cases, questions remain over the effect of these detergents on the integrity of the proteins they solubilize. Moreover, there are many important cases, notably GPCRs, large membrane transporters, and some membrane protein complexes, in which isolating functional protein remains challenging. In order to further our understanding of these fundamentally important molecules, membrane protein biochemists require alternatives to detergents; enter amphipathic polymers. In our laboratories, the polymer of choice is a copolymer of 2:1 styrene: maleic acid (SMA). This has been shown to directly solubilize most biological membranes, to stabilize membrane proteins, and to be amenable to many important biochemical, functional and structural analyses. Most importantly of all, SMA solubilizes lipids rather than proteins, forming SMA lipid particles (SMALPs) in which membrane protein are retained in their native lipid environment and exhibit excellent functional and structural stability. As our understanding of SMA and SMALPs deepens, we have had success in the purification and study of more than 50 membrane proteins with a variety of architectures and sizes, both beta barrels and alpha helical proteins from 40 to 400 kDa in size. Here I present an overview of this work and show the ongoing method developments that we have pursued to broaden the applications of SMA and SMALPs to membrane protein research.
STONEFISH TOXIN DEFINES AN ANCIENT BRANCH OF THE PERFORIN-LIKE SUPERFAMILY

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The lethal factor in stonefish venom is stonustoxin (SNTX), a heterodimeric cytolytic protein that induces cardiovascular collapse in humans and native predators. Here, using X-ray crystallography we make the unexpected finding that SNTX is a pore-forming member of an ancient branch of the Membrane Attack Complex-Perforin/Cholesterol Dependent Cytolysin (MACPF/CDC) superfamily. Stonustoxin comprises two homologous subunits (α and β), each of which comprises an N-terminal pore forming MACPF/CDC domain, a central focal adhesion-targeting domain, a thioredoxin domain and a C-terminal PRYSPRY immune recognition domain. Crucially, structural comparison of these data provide long-sought after near-atomic resolution insights into how MACPF/CDC proteins assemble into pre-pores on the surface of membranes. Further, our analyses reveal that SNTX-like MACPF/CDC proteins are distributed throughout eukaryotic life and play a broader, possibly immune-related function, outside venom.

UNDERSTANDING ARTEMISININ ACTION IN PLASMODIUM FALCIPARUM

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Artemisinin, and derivatives, are activated by free haem, leading to a free radical form which can promiscuously react with parasite proteins, resulting in parasite killing. We have previously demonstrated that artemisinin treatment induces growth retardation and an accumulation of ubiquitinated proteins, indicative of a cellular stress response that involves the ubiquitin/proteasome system. Ubiquitinated proteins are degraded by the proteasome, and accordingly, proteasome inhibitors strongly synergise artemisinin activity against both sensitive and resistant parasites. Resistant parasites, with mutations in the beta-propeller region of the K13 protein, have lower levels of artemisinin-induced ubiquitinated proteins and delayed onset of cell death. To further examine the mechanism by which artemisinin disrupts normal protein metabolism we undertook biochemical analyses of the ubiquitin/proteasome pathway in parasites following drug treatment. Using a fluorescent reporter line targeted for degradation by the proteasome we find that artemisinin treatment results in an accumulation of unfolded protein. This contrasts to proteasome inhibitors, which result in accumulation of folded protein. Defining these disruptions in protein homeostasis has given us insights into the mechanism of action of artemisinins and helps guide our understanding of artemisinin resistance.
SYM-05-01
THE MEDICAL GENOME REFERENCE BANK - DEEP GENOMICS OF A DISEASE-DEPLETED, ELDERLY AUSTRALIAN POPULATION
Pinse M.1, Lacaze P.2, Rath E.1, Kaplan W.1, Andrews D.3, The Arthritis Investigator Group4, The 45 And Up Study Collaborators4, Barr M.1, Thomas D.M.1 and Dinger M.1
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SYM-05-02
INTEGRATING HUMAN EPIDEMIOLOGY AND MOLECULAR BIOLOGY IN THE ERA OF BIG DATA
Makinen V.P.
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SYM-05-03
RNA-SEQUENCING TO DETECT ONCOGENIC FUSION GENES IN PAEDIATRIC CANCER
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SYM-05-04
IDENTIFICATION OF MODIFIER GENES OF TAUOPATHIES
Przybyla M.1, Stevens C.H.1, Mian B.1, Morahan G.1, Ram R.1, Van Der Hoven J.1, Van Hummel A.1,2, Ke Y.D.1, Ittner L.M.1,3 and Van Eersel J.1
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Accumulation of tau protein is a feature of several neurodegenerative diseases including Alzheimer’s disease (AD) and frontotemporal lobar degeneration (FTLD-tau). Furthermore, tau pathology correlates with cognitive decline and neurodegeneration in these diseases. However, very little is known about mechanisms that protect from tau-induced neurodegeneration. Recent studies of familial FTLD-tau and AD suggest that genetic modifiers may provide a new avenue for prevention of neurodegenerative disorders. However, the identification of modifier genes using conventional methods e.g. human studies has been time consuming, costly and difficult. In this study, we used two unique resources to identify modifier gene(s) that protect against tauopathies. First, a new genetic resource called Collaborative Cross (CC); a large panel of recombinant inbred mice, enabling high genetic diversity, rapid mapping and gene identification of multifactorial traits, as occurring in AD and FTLD-tau. The second resource is our established TAU58/2 transgenic mouse, expressing a human FTLD-tau mutation. Those mice develop early-onset disinhibition and muscle degeneration, features found in FTLD-tau and AD patients. We crossed 50’CC strains onto TAU58/2 transgenic mice and assessed functional deficits. One strain showed protection against two traits- disinhibition and weight/ muscle loss. To date we have back-crossed and analysed over 300 mice and maintained both protected traits. Furthermore, by using the mapping power of CC, we have targeted a novel gene on chromosome 8, linked to the protected phenotype. Now we will introduce the identified polymorphism into a susceptible background and show that this new line is protected against TAU58/2 functional deficits.
SYMPOSIA
TUESDAY

SYM-05-05
INTEGRATED IN SILICO AND EXPERIMENTAL ASSESSMENT OF DISEASE-RELEVANCE OF PROTOCADHERIN 19 MISSENSE VARIANTS
Pham D.1,2, Schulz R.1, Kolk C.1, Corbett M.1,2, Epik4 Consortium3, Epilepsy Phenome Genome Project4, Pitson S.1, Petrovski S.1, Pitman M.1 and Gecz J.1,2,5. 1Adelaide Medical School, The University of Adelaide, Adelaide 5005, Australia. 2Robinson Research Institute, The University of Adelaide, Adelaide 5006, Australia. 3Centre for Cancer Biology, University of South Australia, Adelaide 5000, Australia. 4Department of Medicine, Austin Health and Royal Melbourne Hospital, The University of Melbourne, Melbourne 3050, Australia. 5South Australian Health and Medical Research Institute, Adelaide 5000, Australia. https://www.epi4k.org/. http://www.epgp.org.

Mutations in the cell adhesion/estrogen receptor transcription co-repressor molecule protocadherin 19 (PCDH19) cause girls clustering epilepsy (GCE) and is the second most frequent single gene cause of epilepsy. The genetics of this disorder is unusual. PCDH19 is on the X chromosome. Heterozygous females are affected, while hemizygous males are not, contradicting normal X-linked disorders. Males with a postzygotic somatic mutation in PCDH19 are also affected. Cellular mosaicism due to X-chromosome inactivation in females is the likely driver of the disorder. Our research shows that estrogen receptor alpha (ERα) is involved in the pathogenesis of PCDH19-GCE. We performed integrative in silico (25 different bioinformatics tools, protein structure-modelling) and experimental (ERE-LUC reporter assays, RT-qPCR, Western blotting of ERα endogenous gene targets e.g. QR, AKR1C3) evaluation of the functional impact of 31 different PCDH19 missense variants. These included published PCDH19-GCE disease-causing variants (n=9), benign higher frequency (ExAC or gnomAD) variants (n=7) and variants of unknown significance (VUS) (n=15). Out of 31 variants, 21 with protein structure availability were tested using all pathogenicity assessment tools. We achieved accurate prediction for 17/21 variants including 78% (7/9) published PCDH19-GCE, 100% (3/3) frequent variants and 78% (7/9) VUS in the concordance of in silico and experimental results. By integrating selected in silico and experimental tools, we can now significantly improve the interpretation of disease-relevance of PCDH19 missense variants. This is crucial for clinical trials and personalised medicine for PCDH19-GCE patients.

SYM-06-01
APOTOPSIS SIGNAL REGULATING KINASES-LINKING REDOX STRESS TO MAPK SIGNALLING
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Apoptosis signal-regulating kinases (ASK1-3) are apical kinases of the p38 and MAPK kinase pathways. They are activated by diverse stress stimuli, including reactive oxygen species, cytokines, and osmotic stress; however, a molecular understanding of how ASK proteins are controlled remains obscure. Our recent structural studies of ASK proteins have revealed a unique fold that links the ASK1 kinase domain to its redox-sensitive thioredoxin-binding domain. This central regulatory region appears to play two roles: actively priming MKK6, a key ASK1 substrate, for phosphorylation; and bringing the ASK1 kinase and thioredoxin-binding domains into proximity for kinase regulation. We have also used biochemical approaches and mass spectrometry to probe oxidative interplay between ASK1 and thioredoxin. Together, these studies allow us to propose a revised model for understanding regulation of ASK-type kinases by oxidative modification, and demonstrate an unexpected case of autoregulatory scaffolding in mammalian stress-activated MAP kinase signalling.

SYM-06-02
CASPASES SUPPRESS DAMP SIGNALING TRIGGERED BY APOPTOTIC MITCHONDRIA
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The apoptotic caspases are proteases that function at the terminal end of apoptosis, mediating many of the hallmarks of this form of cell death, e.g. membrane blebbing, DNA laddering and phosphatidylserine exposure. For many years caspases were thought to be essential for death, but it is increasingly apparent that they do not instigate the killer event, instead acting to accelerate cellular demise. In doing so, caspases play an important role in preventing apoptotic cells from becoming pro-inflammatory and potentially immunogenic. A prime example is the ability of caspases to prevent dying cells from producing interferon (a potent anti-viral cytokine). During intrinsic apoptosis, BAK/BAX-mediated damage to the mitochondria triggers cytochrome-c release and, subsequently, the efflux of mitochondrial DNA (mtDNA) into the cytoplasm, which, in the absence of caspase activation, triggers the innate anti-viral cGAS/STING-signaling pathway to induce interferon production. In other words, without caspases, a dying cell behaves as if it were virally infected. Exploiting recent advances in microscopy technology, we utilized lattice light-sheet microscopy to document mitochondrial dynamics inside live cells with stunning resolution. This approach allowed us to observe mtDNA release from apoptotic mitochondria for the first time, and in turn provided key insights into the molecular mechanism governing its release.

SYM-06-03
PUMPING-UP CELL DEATH MEDIATED BY SMAC-MIMETICS
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Resistance to chemotherapy is a major problem in cancer treatment and frequently associated with failure of tumor cells to undergo apoptosis. Novel therapies that induce alternative death pathways, such as RIPK3/MLKL dependent necroptosis, may be a new strategy to overcome chemoresistance. The smac-mimetic birinapant, a drug in clinical trials, mimics the interaction between Inhibitor of Apoptosis proteins (IAPs) and Smac/DIABLO, thereby relieving IAP mediated caspase inhibition and promoting apoptosis of cells. Using murine models of Acute Myeloid Leukemia (AML) that recapitulate human disease, and primary human AML cells, we found that these leukemias differ in their sensitivity to birinapant induced apoptosis. Furthermore, our in vitro and in vivo data indicate that prolonged treatment of AML cells with birinapant may lead to resistance. In our studies, we have examined the emergence of resistance in our model systems of AML treated with birinapant and explore strategies by which resistance can be overcome. Our findings reveal new and unexpected combined therapies and biomarkers of response that will be essential for the design of effective clinical trials for birinapant and other Smac-mimetics.
TARGETING SPHINGOSINE KINASE 1 INDUCES MCL-1 DEPENDENT CELL DEATH IN ACUTE MYELOID LEUKAEMIA

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Acute myeloid leukemia (AML) is an aggressive malignancy where despite improvements in conventional chemotherapy and bone marrow transplantation, overall survival remains poor. Sphingosine kinase 1 (SK1) generates the bioactive lipid sphingosine 1-phosphate (S1P) and has established roles in tumor initiation, progression and chemotherapeutic resistance in a wide range of cancers [1]. The role and targeting of SK1 in AML has not been extensively investigated. Here, we show that SK1 is overexpressed and constitutively activated in primary AML patient blasts but not in normal mononuclear cells. Subsequent targeting of SK1 induced caspase-dependent cell death in AML cell lines, primary AML patient blasts, and isolated AML patient leukaemic progenitor/stem cells, with negligible effects on normal bone marrow CD34+ progenitors from healthy donors. Furthermore, administration of SK1 inhibitors to orthotopic AML patient-derived xenografts reduced tumor burden and prolonged overall survival without affecting murine hematopoiesis. SK1 inhibition was associated with reduced signaling from S1P receptor 2, resulting in selective down-regulation of the pro-survival protein Mcl-1. These results support the notion that SK1 is a bone fide therapeutic target for the treatment of AML [2].


GENOME-WIDE ASSOCIATION STUDIES IDENTIFIED CANDIDATE GENES FOR DISEASE RESISTANCE USING WHOLE GENOME RE-SEQUENCING DATA IN CHICKPEA

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Ascochyta blight (AB) is a fungal disease that can significantly reduce chickpea production in Australia and other regions of the world. Sixty-nine chickpea genotypes were sequenced using whole genome re-sequencing (WGRS) resulting in more than 800,000 single nucleotide polymorphisms (SNPs). Population structure analysis revealed two groups of cultivars separated based on their level of ascochyta blight (AB) resistance and narrow genetic diversity in recently released Australian cultivars. Both Fst genome scan and genome-wide association studies (GWAS) identified a ~100kb region on chromosome 4 that was significantly associated with AB resistance. This region was co-located in a large QTL interval of 7Mb~30Mb identified previously in three different mapping populations genotyped at low density with SSR or SNP markers. The ~100kb region has been validated by GWAS in an additional 132 advanced lines with ~140,000 SNPs. Reduced level of nucleotide diversity and the long extent level of linkage disequilibrium also suggested this region may have gone through selective sweeps caused by selection of AB resistance traits in breeding. In total, 12 predicted genes were located in this region including NBS-LRR receptor-like kinase led to amino acid substitutions. One significant SNP located in the coding sequence of NBS-LRR receptor-like kinase led to amino acid substitution. Transcriptional analysis using qPCR showed that some predicted genes were significantly induced in resistance lines after inoculation compared to non-inoculated plants. Phytophthora root rot (PRR) is another important fungal disease in Australia. We have identified several major QTLs using three related RIL mapping populations. Some of the QTLs have been validated using GWAS with 300 advanced lines. An RNAseq approach is currently underway to identify differentially expressed genes involved in PRR resistance.

PHOSPHOLIPID-MEDIATED OLIGOMERIZATION OF DEFENSINS INDUCES FUNGAL AND TUMOUR CELL LYSIS

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Cationic antimicrobial peptides (CAPs) such as defensins are ubiquitously found innate immune molecules that often exhibit broad activity against microbial pathogens and mammalian tumor cells. Many CAPs act at the plasma membrane of cells leading to membrane destabilization and permeabilization. Here we describe a novel cell lysis mechanism for fungal and tumor cells by plant defensins that act via direct binding to the plasma membrane phospholipids phosphatidylidylinositol 4,5-bisphosphate (PIP2) or phosphatidic acid (PA). We have determined the crystal structures of the plant defensins NaD1 bound to PIP2 and NsD7 bound to PA, respectively, revealing distinct oligomeric arrangements. Both NaD1 and NsD7 form dimers that cooperatively bind the anionic head groups of PIP2 or PA via unique "cationic grip" configurations and assemble into oligomeric fibrils. We have now determined the structure of NaD1 in complex with PA. NaD1:PA crystalized as a large 20-meric complex that adopts a nearly flat overall shape, forming a defensin carpet with a strikingly different topology compared to previously determined defensin:phospholipid structures. These observations identify a conserved innate recognition system by defensins for direct binding of phospholipid that permeabilize cells via a novel membrane disrupting mechanism, and provide a first glimpse of the structural details of molecular events at the contact point between defensins and membranes.

SPHINGOSINE KINASES AS THERAPEUTIC TARGETS IN GLOIOBLASTOMA MULTIFORME

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Glioblastoma Multiforme (GBM) is the most commonly diagnosed brain tumour in adults. Current standard treatment consists of surgical resection followed by radiation and chemotherapy using the DNA alkylating agent Temozolomide (TMZ). Despite aggressive therapy, current median survival is approximately 15 months, therefore there is a desperate need to identify new effective targeted therapies for this cancer. Sphingosine kinases (SKs) are intracellular signalling enzymes which have been implicated in cancer initiation, progression and chemotherapeutic resistance. Sphingosine kinase 1 (SK1) is known to be elevated in the tumours of GBM patients, whilst the role of SK2 in GBM is less clear. We have shown that our novel SK1 inhibitor MP-A08 and a selective SK2 inhibitor K145 are able to reduce tumour cell growth in U251, U87 and T98G GBM cell lines. Furthermore, in vivo, this SK inhibitor reduced the growth of human GBM cell line xenografts. Our current studies utilise low passage patient-derived GBM cell lines which provide a physiologically-relevant resource to investigate the targeting of SKs in GBM, as they more closely reflect primary GBM tumours compared to laboratory-adapted GBM cell lines. These patient-derived cells represent the four molecular subtypes of GBM, allowing examination of the sensitivities of these subtypes to SK inhibitors alone, and in combination with chemotherapy and/or radiotherapy. This information will stratify which GBM tumours are likely to respond to SK-based therapies, and may provide attractive therapeutic options for subtypes which currently show no response to therapy.
SYM-07-03
FUNCTIONAL CHARACTERISATION AND STRATEGIES FOR DEPLOYMENT OF RESISTANCE GENES AGAINST THE MAJOR BIOTROPHIC PATHOGENS OF CULTIVATED WINEGRAPE

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The most economically important diseases of grapevine cultivation worldwide are caused by the biotrophic pathogens powdery mildew (Erysiphe necator syn. Uncinula necator) and downy mildew (Plasmopara viticola). Currently, grapegrowers rely on the frequent application of fungicides to minimise the potentially devastating impact of these pathogens on grape yield and quality. We have mapped and cloned two resistance genes from the wild North American grapevine species Muscadinia rotundifolia that encode Toll/Interleukin-1 receptor (TIR) - nucleotide-binding (NB) - leucine-rich repeat domain (LRR) proteins. These two genes, designated Resistance to Uncinula necator (RUN1) and Resistance to Plasmopara viticola (RPV1), confer strong resistance to these pathogens following either genetic transformation or introgression into highly susceptible premium winegrape varieties. Functional characterisation of the TIR and LRR domains of RPV1 confirm that specificity of effector recognition is dictated by the LRR region, while the TIR domain is required for signal transduction. Both RUN1 and RPV1 confer resistance to multiple powdery and downy mildew isolates from France, North America and Australia. However, a single powdery mildew isolate collected from the same sample-set, we propose that cv. Clipper could adopt a more effective mechanism to cope with the ambient high salt conditions. More than 90% transcript-mapping efficiency achieved, limma-based generalized linear models were then applied to statistically assess the impact of high salinity in different cultivars of barley, de novo transcriptome assemblies of a malting cultivar (cv. Clipper) and a landrace (cv. Sahara) with known contrasting responses to salinity during their seedling stage were generated. However, stemmed from the relatively low level of functional annotations (~23%) of the assemblies in the previous study, gene-clusters enriched and differential transcripts identified upon salt stress were not conclusive. In this study, taking advantage of the newly available barley reference genome (cv. Morex) and energy applications.

SYM-07-04
THE GENETIC BASIS OF RESISTANCE IN BARLEY TO DIVERSE Puccinia STRIIFORMIS ISOLATES

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The genetic basis of resistance to stripe rust (P. striiformis) in barley is not well understood. Barley is infected by the adapted pathogen Puccinia striiformis f. sp. hordei (Psh), and is an intermediate or near nonhost to the formae speciales adapted to wheat [f. sp. tritici (Pst)] and to barley grass [f. sp. pseudo-hordei (Psph)]. To determine the genetic basis of resistance to P. striiformis in barley, we developed a recombinant inbred line (RIL) population using a P. striiformis-susceptible accession (Biosaline-19) and the immune cultivar Pompadour, which carries adult plant resistance (APR) to leaf rust (Rph20). The immunity in Pompadour at the seedling stage to four diverse P. striiformis isolates was due to resistance QTL on chromosomes 1H, 2H, 4H, 5H and 7H with both overlapping and distinct specificities. The presence of isolate specificity was further supported using a histological approach, where RILs were identified with ranging responses to the three P. striiformis isolates. The population was also phenotyped in the field for response to Psh, Psph and P. hordei (barley leaf rust pathogen). Seedling-susceptible RILs to Psph were resistant in the field suggesting involvement of APR. Additional QTLs were identified on chromosome 7H at the same genetic position as Rph23 (APR to leaf rust) suggesting either pleiotropic resistance or the involvement of a gene closely linked to or allelic with Rph23. Unlike many pleiotropic APR genes identified and isolated in wheat, our data suggest that Rph20 does not confer resistance to the P. striiformis isolates used in this study.

SYM-07-05
SALINITY-INDUCED ALTERATION OF ROOT-OMICS IN BARLEY (Hordeum Vulgare L.)

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Due to increases in soil salinity in the temperate region optimal for cultivation, barley (Hordeum vulgare L.) has been suffered from substantial yield loss in Australia during the last decade. To understand the impact of high salinity in different cultivars of barley, de novo transcriptome assemblies of a malting cultivar (cv. Clipper) and a landrace (cv. Sahara) with known contrasting responses to salinity during their seedling stage were generated. However, stemmed from the relatively low level of functional annotations (~23%) of the assemblies in the previous study, gene-clusters enriched and differential transcripts identified upon salt stress were not conclusive. In this study, taking advantage of the newly available barley reference genome (cv. Morex) and energy applications.

SYM-08-01
INTERFACING BIOMOLECULES WITH NANOMATERIALS: STRUCTURE AND FUNCTION AT THE ATOMIC-SCALE

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An in-depth appreciation of how to control the interaction of peptides with materials interfaces, including nanoparticles, at the molecular level, will advance application areas such as self-organised metamaterials for photonics and plasmonics, biosensing, catalysis, energy generation and harvesting, and nano-medicine. Exploitation of materials-selective binding of biomolecules is key to success in these areas; i.e. by realising preferential adsorption of a biomolecule onto one materials composition over another, one materials facet over another, or one materials polymorph over another. Structural characterisation of the surface-adsorbed biomolecules is essential for establishing the required structure/property relationships in these systems, but challenging to accomplish via experimental approaches alone. In partnership with experimental characterisation, molecular simulations can bring complementary insights into the origins of this selectivity, and suggest routes to manipulating these phenomena for realising new types of hybrid materials. Our team specialise in the development and deployment of interfacial force-fields and molecular simulation techniques for the purpose of elucidating these insights at biomolecule/materials interfaces. In this contribution I will outline our developments and applications of advanced molecular simulation approaches for investigating these challenging interfacial systems, and our findings for manipulating the adsorption of biomolecules at the aqueous interface for bio/nano applications in e.g. sensing, catalysis and energy applications.
SYM-08-02
MECHANISTIC STUDIES OF CARBOHYDRATE-ACTIVE ENZYMES AND IMPLICATION FOR INHIBITOR DESIGN

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Carbohydrate-active enzymes (CAZymes) are families of essential and structurally related enzymes, which catalyse the creation, modification, and degradation of glycosidic bonds in carbohydrates to maintain essentially all kingdoms of life. CAZymes play a key role in many biological processes underpinning human health and diseases (e.g., cancer, diabetes, Alzheimer’s diseases, AIDS) and have thus emerged as important drug targets in the fight against pathogenesis. The realisation of the full potential of CAZymes remains a significant challenge, relying on a deeper understanding of the molecular mechanisms of catalysis. Considering numerous unsettled questions in the literature, while with a large amount of structural, kinetic, and mutagenesis data available for CAZymes, there is a pressing need and an abundant opportunity for collaborative computational and experimental investigations with the aim to unlock the secrets of CAZyme catalysis at an atomic level. In this talk, I will briefly talk about our recent work on computational studies of CAZyme catalysis including determination of catalytically competent protonation states and exploring different reaction pathways. Implication for inhibitor design by mimicking the transition state is also illustrated for glycosyltransferases. The challenges for such studies will be noted and finally an outlook for future directions will be provided.

SYM-08-03
THE TEMPERATURE-DEPENDENT CONFORMATIONAL DYNAMICS OF THE CYSTEINE-RICH SPIDER PEPTIDE GOMESIN

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Gomesin is an 18-residue peptide isolated from the haemocytes of the Brazilian spider Acanthoscurria gomesian that forms a two-stranded beta-sheet connected by a beta-turn and stabilised by two disulphide bonds. The peptide shows potent antimicrobial and antitumor activities employing a membrane-permeabilising mechanism resulting in cell lysis. With a view towards establishing a structure-activity relationship of the peptide we carried out a series of MD simulations of the Gomesin and Gomesin variants in solution. A first set of simulations at 298K revealed backbone flexibility that is uncharacteristic for a cysteine-rich peptide. The solution structure of gomesin was determined using data from NMR spectra collected at 278K. For validation, simulations were repeated at 278K and the chemical shifts calculated from MD simulations show good agreement with experimental data. Comparison of the peptide conformations at 278K, 298K and 310K suggest that unlike most cysteine-rich peptide gomesins shows a significant increase in backbone flexibility with increasing temperature and at higher temperatures adopts conformations not observed at lower temperatures. This is consistent with chemical shift data from 298K that indicates a loss of secondary structure compared to 278K. However, the extent of this temperature effect and the conformational ensemble sampled by the peptide is force field dependent. This introduces challenges for modelling gomesin not usually encountered in cysteine-rich spider peptides.

SYM-08-04
MHC-I PEPTIDES GET OUT OF THE GROOVE AND ENABLE A NOVEL MECHANISM OF HIV-1 ESCAPE

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Major histocompatibility complex class I (MHC-I) molecules play a crucial role in both adaptive and innate immunity through the capture of intracellular peptides for presentation to T cells and natural killer (NK) cells. In MHC-I, the closed ends of the peptide binding groove result in intracellular peptides for presentation to T cells and natural killer (NK) cells. In MHC-I, the closed ends of the peptide binding groove result in bound peptides tethered at both their N and C termini. Here we show that 298K, the HLA-B*57:01 peptide repertoire comprises N-terminally extended sets characterised by a common motif at position 1 (P1) to P2. Structures of HLA-B*57:01 presenting N-terminally extended peptides, including the immunodominant HIV-1 Gag epitope T10 (TSTLQEQIGW), showed that the N-terminus protrudes from the peptide-binding groove. The common CD8+ T cell response escape mutant TSNLQEQIGW bound HLA-B*57:01 canonically, adopting a dramatically different, register-shifted conformation compared with the TW10 peptide. This register shift of the T3N escape mutant epitope in the HLA binding groove was also shown to affect recognition by killer cell immunoglobulin-like receptor (KIR) 3DL1 expressed on NK cells. Structural characterisation of both KIR3DL1-HLA complexes allowed us to determine that this register shift primarily affects contacts between KIR3DL1 and the bound peptide. We thus define a previously uncharacterised feature of the human leukocyte antigen class I (HLA-I) immunopeptidome, additionally highlighting the peptide dependence of KIR-HLA interactions and the their implications for viral immune escape. We further suggest that recognition of the HLA-B*57:01-TW10 epitope is governed by a ‘molecular tension’ between the adaptive and innate immune systems.

SYM-08-05
3D PROTEIN STRUCTURE DETERMINATION USING LANTHANIDE PROBES

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Protein 3D structure determination using computational/experimental hybrid methods allows smart information usage by combining minimal sets of structural data from a range of biochemical and biophysical experiments with molecular modelling. Paramagnetic lanthanide ions are particularly attractive probes to generate such data by NMR spectroscopy, because they provide structural restraints which are orientation dependent and long-range. By combining minimal sets of structural data from a range of biochemical and biophysical experiments with molecular modelling. Paramagnetic lanthanide ions are particularly attractive probes to generate such data by NMR spectroscopy, because they provide structural restraints which are orientation dependent and long-range. By combining minimal sets of structural data from a range of biochemical and biophysical experiments with molecular modelling. Paramagnetic lanthanide ions are particularly attractive probes to generate such data by NMR spectroscopy, because they provide structural restraints which are orientation dependent and long-range (up to 40 Angstrom from the metal centre) due to the strong interaction of the unpaired electron with nuclei in the protein. The focus of this talk will be on protein 3D structure determination by assembling super-secondary structure motifs with the help of pseudocontact shift (PCS) restraints for backbone amide protons, where the PCSs are produced from different metal centres. I will show successes and pitfalls of the new assembly algorithm, and discuss how the sparsity of data affects model quality.
SYM-09-01

AGEING DISGRACEFULLY: REFINING THE MUTAGENIC INFLUENCE OF 5MC

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The instability of 5mC has shaped the human genome on an evolutionary timescale. Hundreds of deamination events occur each day in every nucleated cell in the body, requiring a coordinated and efficient repair network. Here we reveal the consequences of disrupting this finely-tuned repair network. We describe a novel cancer predisposition syndrome with a near complete dependence on 5mC as the mutagenic stimulus. Despite their being millions of 5mC residues spread throughout the genome, the three individuals under study all developed the same type of cancer - acute myeloid leukaemia. Even more surprising, the cancers share a common set of driver mutations, and even acquire the mutations with a similar chronology, reflecting a conserved mutagenic cascade. Our results shed new light on how the gradual accumulation of DNA damage can profoundly impact the biology of blood stem cells and raises new questions regarding the intricate connection between DNA methylation and repair.

SYM-09-02

GATTACA 20 YEARS ON: JUST HOW CLOSE ARE WE?

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With the rapid progress in genomic technology, the concept of genetic testing in a newborn baby to predict their future health is closer than ever before. This technology is increasingly used to diagnose individuals with rare, single gene disorders encompassing a wide range of genetic diseases. However, interpretation of genetic variation still remains a challenge in these individuals. How then, can one hope to interpret genetic variation in a newborn in the absence of a disease phenotype? The societal and ethical implications of this technology are significant and are particularly challenging when applied to healthy individuals. Using examples of Mendelian disease diagnoses through to sequencing of healthy adults, I will explore what is currently possible with genomic testing and what the future may hold. Will life imitate art? Can our genetics reliably predict our future?

SYM-09-03

GENOMICS AT THE COAL FACE: A CLINICIAN’S PERSPECTIVE OF THE GENOMIC REVOLUTION

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Recent advances in high-throughput DNA sequencing combined with databases that allow sharing of clinical and genotypic information between clinicians and researchers, such as those in the Matchmaker Exchange hub, have changed the landscape of research into the cause of Mendelian disorders. This has led to an acceleration of disease variant discovery and validation and an exponential increase in clinical diagnoses. As a clinician keeping up with the pace of change can be exhausting but exhilarating. A major challenge is how to bring the families we see along with us on this journey. How can we make our discoveries meaningful to families and ultimately what value do families place on these diagnoses? I will present collaborative work that demonstrates how high-throughput sequencing can act as a springboard to delineate novel pathophysiological mechanisms for new Mendelian neurocognitive disorders, in particular intellectual disability, autism and epilepsy, and our endeavours to make this work clinically useful. No researcher is an island, and I will show how rewarding it can be to include families as critical members of the clinical research team, alongside biologists, bioinformaticians, pathologists, health economists, psychologists and clinicians.

SYM-09-04

INTEGRATED ‘OMICS DISCOVERS CLINICALLY RELEVANT COPY NUMBER AND SEQUENCE VARIANTS IN CEREBRAL PALSY

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Cerebral palsy (CP) describes a group of heterogeneous disorders affecting movement and posture that are caused by a non-progressive lesion or abnormality in the pre or postnatal brain. Comorbidity of CP and disorders with predominantly genetic origin such as intellectual disability and epilepsy suggests that a similar genetic contribution to CP is likely. Our initial exome study of 98 trios, yielded a conservative molecular diagnostic rate of 14% however a further 44% had variants of unknown significance. In this study, interrogation of existing exome data from 198 probands, in combination with RNA-Seq from 182 patient derived lymphoblastoid cell lines (LCL) yielded an improved interpretation of variants of unknown significance and facilitated discovery of copy number variants. We discovered 6 deletions and 8 duplications in the exome data that were validated by Illuminia Infinium CytosNP-850 arrays. Expression of consecutive genes in each CNV was altered consistent with gene dosage. Outlier expression levels detected in other samples provided validation of premature termination or splice site defects in a further 9 genes. Integrated analysis of our exome, RNA Seq and SNP array data brings our current likely pathogenic variant rate to 25% for this cohort supporting a significant and previously unappreciated contribution of genetics to CP. Differentially expressed genes in the whole cohort were enriched for trophic signalling pathways. Weighted gene coexpression analysis also revealed the genetic landscape of CP shares significant overlap with genes implicated in autism spectrum disorders suggesting a possible link between these two neurodevelopmental disorders with known environmental and genetic components.
SYM-09-05
IDENTIFICATION AND ANALYSIS OF THREE NOVEL ENHANCERS OF HUMAN SOX9: IMPLICATIONS FOR DISORDERS OF SEX DEVELOPMENT

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Disorders of Sex Development (DSDs) encompass a wide spectrum of conditions and often manifest with atypical gonads or genitalia. The majority of DSD patients cannot be given an accurate diagnosis, which severely compromises their clinical management. While mutations in coding regions of gonad genes have been important in understanding the aetiology of DSD little attention has focused on the regulatory regions of gonad genes. Recent reports by us and others of 46,XX testicular DSD patients with duplications and 46,XY gonadal dysgenesis patients carrying deletions upstream of SOX9 suggest the presence of gonad specific enhancers. Investigating the overlap between these patients has led to the discovery of novel lests specific regulatory regions for SOX9. Using a comprehensive tiling luciferase assay and bioinformatic approaches, we have identified three novel enhancers upstream of SOX9. Enhancers that showed the strongest activity in vitro were used to generate transgenic mice. The enhancers showed expression in embryonic mouse gonads at the time of gonad differentiation. Analysis of these enhancers showed that in response to the transcription factors SF1/SPRY and SF1/SOX9 they recapitulate the initiation, upregulation and maintenance of SOX9 expression exhibited during gonad differentiation. Our results strongly suggest that deletions or duplications (CNVs) of these enhancers may lead to DSD.

SYM-10-02
STEM CELL MIGRATION AND MECHANOTRANSDUCTION ON LINEAR STIFFNESS GRADIENT HYDROGELS


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The spatial presentation of mechanical information is a key parameter for cell behavior. We have developed a method of polymerization control in which the differential diffusion distance of unreacted cross-linker and monomer is preprogrammed to vary the stiffness gradient of the polymer matrix. This simple, low-cost method was used to produce polyacrylamide hydrogels with stiffness gradients of 0.5, 1.7, 2.9, 4.5, 6.8, and 8.2 kPa/mm, spanning the in vivo physiological and pathological mechanical landscape. Importantly, three of these stiffness gradients were found to be nondorutocatic for human adipose-derived stem cells (hASCs), allowing the presentation of a continuous range of stiffnesses in a single well without the confounding effect of differential cell migration. Using these nondorutocatic stiffness gradients, stiffness-dependent hASC morphology, migration, and differentiation were studied. Finally, the mechanosensitive proteins YAP, Lamin A/C, Lamin B, MRTF-A, and MRTF-B were analyzed on these gradients, providing higher-resolution data on stiffness-dependent expression and localization.

SYM-10-03
MECHANOSENSING IN CHONDROCYTES VIA MECHANICALLY GATED ION CHANNELS

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The joints of mammals are lined with cartilage, comprised of individual chondrocytes embedded in a specialized extracellular matrix. Chondrocytes experience a complex mechanical environment and respond to changing mechanical loads in order to maintain cartilage homeostasis. At the molecular scale, it has long been postulated that mechanosensitive (MS) ion channels are of functional importance in chondrocyte mechanotransduction. In order to directly investigate whether MS channels could be detected in chondrocytes we employed elastomeric pillar arrays to mimic molecular-scale stimuli applied to chondrocytes via their connections to the substrate. We additionally used high speed pressure clamp to investigate whether MS channel activity could also be detected in response to membrane stretch. MS ion channel activity was measured in response to both types of stimuli, however the molecules mediating these currents differed. Both TRPV4 and PIEZO1 channels contribute to currents activated by stimuli applied at cell-substrate contacts but only PIEZO1 mediates stretch-activated currents. These data demonstrate that there are separate, but overlapping, mechanoelectrical transduction pathways in chondrocytes. Thus, at the molecular level, chondrocytes respond differentially to distinct mechanical stimuli.
SYM-10-04
P2X4 CONTROLS MECHANO-TRANSDUCTION OF BLOOD FLOW IN HUMAN MONOCYTES
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In arteries, circulating leukocytes including monocytes are exposed to directional flow dynamic forces that initiate inflammatory vascular reactions. The exact role of P2X4 receptor in blood cells passing through the calcified valve leaflets is exposed to the pathological magnitude of shear stress, and depending on the degree of obstruction can experience shear stress levels ten times higher than their physiological level. Using a combination of TIRF microscopy and Ca2+ imaging, we have found that shear stress increases the localised Ca2+ influx into the cytosolic domain of plasma membrane, and the increase in [Ca2+]i is regulated via an influx of Ca2+ through the ATP-gated purinergic receptor, P2X4. We have also found that shear stress sensitises the response of primary murine monocytes to ATP. The increase in [Ca2+]i is associated with an influx of monocytes to different concentrations of ATP. This process is controlled via an influx of Ca2+. The ATP-gated purinergic receptor, P2X4, is expressed in vascular smooth muscle cells and monocytes and is therefore an important player in the response of these cells to shear stress.

SYM-10-05
THE MECHANOSENSOR YAP DRIVES CUTANEOUS TYPE 2 INFLAMMATION AND ECZEMA DEVELOPMENT
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One in five children in the Western world is affected by eczema. This allergic skin disease develops as result of intrinsic epidermal barrier defects, which drive a local and systemic 'type 2' immune response, resulting in a pathological cycle of itching, scratching and inflammation, and eventually the potentiation of allergic sensitisation. The exact mechanism of how epidermal barrier dysfunction, itch and type 2 inflammation connect at the molecular level remains poorly understood. Yes-associated protein (YAP) is a mechanosensor that responds to mechanical stimuli to control tissue homeostasis. In this project, transcriptomics analysis identified approximately 2000 differentially expressed genes in YAP2-SSA-ΔC skin with increased nuclear YAP activity in the basal epidermis. A large number of these genes encode proteins involved in type 2 immune response, including the cytokine IL-33, a key driver of type 2 inflammation. Furthermore, we also found increased FPR2 levels in the presence of C5a in the murine model of the human muscle disease.

SYM-11-01
THE INOSITOL POLYPHOSPHATE 5-PHOSPHATASE SKIP (INPP5K) IS ESSENTIAL FOR SKELETAL MUSCLE INTEGRITY AND FUNCTION
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Phosphoinositol signaling lipids interact with a plethora of effectors to regulate cell proliferation and survival, vesicular trafficking, actin dynamics, and metabolism. Phosphoinositol 3-kinase (PI3K) generates P(3,4,5)P3, which activates many effectors. In skeletal muscle PI3K/Akt/mTOR signaling promotes muscle hypertrophy, post-prandial glucose uptake, and autophagy inhibition. Many inositol polyphosphates (5-phosphatases) degrade PI(3,4,5)P3 and suppress Akt signaling. The 5-phosphatase SKIP (INPP5K) is expressed in skeletal muscle and inhibits growth factor-stimulated Akt/mTOR signaling. Heterozygous Skip knockout mice exhibit increased muscle mass, insulin hypersensitivity and resistance to high-fat diet-induced obesity. However, two recent studies have identified human loss-of-function INPP5K mutations cause congenital muscular dystrophy characterized by short stature, and muscle weakness, associated with early-onset cataracts and mild cognitive impairment. Muscle biopsies from affected individuals show necrotic/degenerative muscle fibres, regenerating fibres, centralised nuclei, vacuolated fibres and fibrosis. Despite these studies, the mechanism of disease remains unknown. To determine the role SKIP/INPP5K plays in skeletal muscle, we have generated a muscle specific Skip knockout mouse. Interestingly, Skip-null murine muscle phenocopies human INPP5K mutant muscle with early-onset, progressive and degenerative muscle disease, characterized by muscle degeneration, loss of muscle mass and significant muscle weakness. Murine Skip-null muscle shares a similar histopathological profile to human muscle with INPP5K mutations, making this an ideal murine model of the human muscle disease. Studies into the molecular mechanism of disease are currently ongoing.

SYM-11-02
IDENTIFICATION OF A NOVEL REGULATOR OF CHOLESTEROL METABOLISM USING AN INTEGRATIVE SYSTEMS APPROACH
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Background: The liver controls numerous pathways central to the maintenance of whole body lipid levels. Disruption of these pathways increases the risk of diseases including insulin resistance and cardiovascular disease. Thus, we need a greater understanding of the pathways regulating hepatic lipid metabolism. Methods: We utilised our exclusive access to a panel of >100 genetically inbred mouse strains known as the hybrid mouse diversity panel (HMDP) at UCLA. We took a trans-omics approach to analyse mouse livers, integrating genetics, phenomics, lipidomics (>300 species) and proteomics (>5000) to identify novel pathways involved in regulating hepatic lipid metabolism. Results: An 11-fold difference in hepatic cholesterol ester (CE) levels was observed across strains in the absence of dietary intervention. In addition to identifying known bone fide regulators of cholesterol metabolism, we identified novel proteins associated with hepatic CE accumulation. One such protein that we named novel membrane protein (NMP; p<0.0001) was regulated inversely with cholesterol levels. Ecopic expression of NMP in vitro and in vivo was associated with a significant upregulation of cholesterol ester levels. Moreover, NMP was associated with CVD and plasma cholesterol and LDL levels in in-vivos from the San Antonio Family Heart Study. Conclusions: We have established a high-resolution trans-omics network for the identification of novel regulators of hepatic lipid metabolism.
**SYM-11-03**

**LIPIDIC PATHWAY REQUIRED FOR PLANT POLLEN DEVELOPMENT**

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The pollen wall, a specialized extracellular cell wall matrix, surrounds male gametophytes and is essential for plant reproduction. Revealing the molecular controls underlying the synthesis and polymerization of the lipidic precursors of pollen wall has been a major research focus. Herein, I will present recent identified genetic and biochemical genes controlling pollen wall development plants, particularly the genes associated with the biosynthesis, transport, and assembly of various lipidic precursors of pollen wall components. I will discuss the conserved and divergent aspects of these genes and their regulation.

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**SYM-11-05**

**A CONSERVED DEGRON REGULATES THE CHOLESTEROL-MEDIATED TURNOVER OF HUMAN SQUALENE MONOOXYGENASE, A RATE-LIMITING ENZYME IN CHOLESTEROL SYNTHESIS**

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Cholesterol biosynthesis is tightly controlled by multiple mechanisms to regulate cellular cholesterol levels. Squalene monoxygenase (SM) is the second rate-limiting enzyme in cholesterol biosynthesis and is regulated both transcriptionally and post-translationally. Post-translationally, SM undergoes cholesterol-dependent proteasomal degradation when cholesterol is in excess. The first 100 amino acids of SM (designated SM N100) are necessary for this degradation process, and represent the shortest cholesterol-regulated degron identified to date. However, the fundamental intrinsic characteristics of this degron remain unknown. To pinpoint the residues, motifs, and regions in human SM N100 required for its cholesterol-mediated regulation, we utilized four approaches: (1) truncation mutations, (2) systematic alanine-scanning mutagenesis, (3) comparative biology and (4) identification of degron elements based on conservation of SM N100 with known degrons. We identified a 20-residue region in the second half of SM N100 required for cholesterol-mediated SM turnover. Furthermore, we found that deletion of a predicted amphipathic helix within this 20-residue region ablates this cholesterol-mediated turnover. Moreover, point mutations of SM based on the key degron residues in a yeast degron, Deg1, blunted the cholesterol-mediated SM turnover. Of note, the amino acid residues of SM N100 and Deg1 were 42% similar. These findings shed new light on the regulation of a key cholesterol synthesis enzyme and highlight the conservation of critical degron features from yeast to humans.

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**SYM-11-04**

**COORDINATING TGN/ENDOSOMAL LIPID METABOLISM WITH CELLULAR QUIESCENCE**

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The Golgi complex constitutes a central way station of the eukaryotic secretory pathway, an intricate network of organelles engaged in control of membrane trafficking and the processing of various cellular components. Historically, functional insight into the mechanisms of vesicular transport are of a largely protein centric focus. However, the critical role that lipids play in regulating vesicle trafficking has been increasingly recognised. Lipid exchange proteins play crucial roles in coordinating lipid metabolism with membrane trafficking in TGN/endosomes. This circuit is controlled by opposing actions of two families of lipid-exchange protein; the pro-trafficking PtdIns/PtdCho transfer proteins (PITP) and the antagonistic short oxysterol binding protein related proteins (ORP-S). It is also now becoming appreciated that such an interface plays an important role in linking secretory pathway function with cell proliferation. To this effect a role for ORP-S proteins in integrating TGN/endosomal lipid signalling with the cell cycle will be discussed and classify ORP-S proteins as novel stage-specific inhibitors of cell cycle progression in eukaryotes.

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**SYM-12-01**

**DETERMINING THE SITE OF ACTION OF STRIGOLACTONES DURING NODULATION**

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Strigolactones (SLs) influence the ability of legumes to associate with nitrogen-fixing bacteria. In this study we determine the precise stage at which SLs influence nodulation. We show that SLs promote infection thread formation, as a null SL-deficient pea (*Pisum sativum* L.) mutant forms significantly less infection threads than wild type plants and this reduction can be overcome by the application of the synthetic SL GR24. We found no evidence that SLs influence physical events in the plant before or after infection thread formation, since SL-deficient plants displayed a similar ability to induce root hair curling in response to rhizobia or Nod factors and SL-deficient nodules appear to fix nitrogen at a similar rate to wild type plants. In contrast, a SL receptor mutant displayed no decrease in infection thread formation or nodule number, suggesting SL-deficiency may influence the bacterial partner. We found this influence of SL-deficiency was not due to altered flavonoid exudation or ability of root exudates to stimulate bacterial growth. The influence of SL-deficiency on infection thread formation was accompanied by reduced expression of some early nodulation (ENOD) genes. Importantly, SL synthesis is down-regulated by mutations in genes of the Nod factor signaling pathway and this requires the downstream transcription factor NSP2 but not NIN. This, together with the fact that the expression of certain SL biosynthesis genes can be elevated in response to rhizobia/Nod factors suggests that Nod factors may induce SL biosynthesis. SLs appear to influence nodulation independently of ethylene action, as SL-deficient and ethylene insensitive double mutant plants display essentially additive phenotypes and we found no evidence that SLs influence ethylene synthesis or vice versa.
SYM-12-02
UNTANGLED THE FACTORS DRIVING NUTRIENT EXCHANGE IN ECTOMYCORRHIZAL SYMBIOSES
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The root tips of most major forest trees are colonised by ectomycorrhizal (ECM) fungi, beneficial microbes that exchange growth-limiting nutrients such as nitrogen (N) for plant photosynthate (C). While this relationship is essential for forest health and sustainability, quantifying the actual benefit of ECM fungi to the plant is complicated and presents a challenge when evaluating below-ground microbe contributions to ecosystem function. To begin to untangle the factors controlling C for N exchange between plants and their associated ECM fungi, we use controlled microcosm environments and isotopic labelling to trace bi-directional nutrient flow between Eucalyptus grandis and Pisolithus isolates. We consider the effect of fungal genotype, competition and substrate nutrient availability on root colonisation and the nutrients traded between E. grandis and Pisolithus. We show that nutrient flow is not simply related to the number of root tips that are colonised - in fact, in many instances the highest levels of colonisation resulted in the least benefit to the plant host. Transcriptomic analysis of mycorrhizal root tips under these different conditions is used to identify some of the pathways and mechanisms behind these observations. We particularly consider potential mechanisms by which the tree host might protect its own ‘interests’ in these interactions should the colonising fungus not prove to be beneficial to the host.

SYM-12-03
MYCORRHIZAL COMMUNITIES AND PHENOTYPES IN RELATION TO ROOT MORPHOLOGY AND GROWTH STRATEGIES OF BRACHYPODIUM DISTACHYON
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Inoculation with arbuscular mycorrhizal (AM) fungi has been demonstrated to substantially enhance plant vigour. However, these results are often difficult to replicate due to complex interactions among the plant and fungal genotypes as well as the local environment. The context dependence of these interactions is a major factor limiting exploitation of AM fungi in agriculture, restoration, and remediation. Our work aims to identify plant and fungal traits that better predict how plants benefit from their AM fungal partners in the presence of important environmental stressors – specifically low nutrient availability, drought, high salinity, and pathogen infection. Here, we describe a series of phenotyping experiments using the grass Brachypodium distachyon, a genomic model for cereal crops. These experiments show that AM fungal communities differ between plants depending on the accession of B. distachyon and within a root system depending on the type of root (seminal, coleoptile nodal, or leaf nodal), but that these differences are small compared to other ecologically important drivers associated with soil. Inoculation with AM fungi did not lead to substantial differences in root morphology for B. distachyon, contrary to expectations, with the exception of a reduction in frequency of coleoptile nodal roots. We also show that growth responses to inoculation with AM fungi depend not only on nutrient limitation but also the extent that the host strategically allocates resources to growth under conditions of nitrogen or phosphorus limitation. These observations are valuable to direct future work on understanding the roles that AM fungi play during interactions with grasses, potentially leading to improved varietal selection and productivity gains, particularly in marginal environments.

SYM-12-04
RHIZOREMEDIATION OF RESIDUAL SULFONUYLUREA HERBICIDES IN AGRICULTURAL SOILS USING LENS CULINARIS AND A COMMERCIAL SUPPLEMENT
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Sulfonylureas are a popular herbicide used today for controlling weeds. While beneficial for this purpose they present a persistent problem in agricultural areas with treated areas proving detrimental for successive crops. This study assessed the phytoremediative properties of lentils (Lens culinaris) grown in un-contaminated and chlorosulfuron-contaminated soil, with and without the addition of a growth supplement, PulseAider™. The results show that in the presence of lentils enhanced the degradation of chlorosulfuron and this degradation rate significantly increased when the PulseAider™ supplement was included during seed sowing. The PulseAider™ also significantly increased shoot and root biomass, root branching and nodule number under control conditions. While this wasn’t so for plant grown in contaminated soils, the PulseAider™ supplement did seem to alter root branching and morphology. Most Probable Number (MPN) assays showed that soil treated with PulseAider™ showed increased numbers of potential chlorosulfuron-degrading bacteria, although this was found to be significant only in the control soil. Sequencing of the 16S ribosomal gene showed the presence of Pseudomonas fluorescens bacterial species which is a known chlorosulfuron-degrading bacterium. This study is one of the first to address the remediation of residual sulfonylurea herbicides and offers an economically feasible solution that may have an impact on global food security.

SYM-12-05
AN IMAGE ANALYSIS SUITE FOR ROOT PHENOTYPING
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In this talk a software suite for analysing images of plant roots will be presented. The suite is comprised of three tools, RootAnalyzer, RootTips and RootGraph, each of which is focused on different types of root images. RootAnalyzer is a fully automated tool for efficiently extracting and analyzing anatomical traits from root cross section images. RootAnalyzer segments the plant root from the image’s background, classifies and characterizes the cortex, stele, endodermis and metaxylem, and produces statistics about the morphological properties of the root cells and tissues. RootTips is a system for the fully automated detection and classification of root tips in root images obtained either by 2D flat bed scanning or in situ imaging. The software provides a robust, efficient and accurate means of phenotyping of roots, by detecting individual root tips and classifying them as belonging to a primary or lateral root. RootGraph is a novel, fully automated and robust approach for the detailed characterization of root traits, based on a graph optimization process. The scheme, firstly, distinguishes primary roots from lateral roots and, secondly, quantifies a broad spectrum of root traits. The program associates lateral roots and their properties with the specific primary root from which the laterals emerge.
Cereal crops such as wheat and rice are rich sources of carbohydrate yet contain low quantities of many essential micronutrients including iron (Fe), zinc and provitamin A. Human Fe deficiency is the most common nutritional disorder in the world, affecting more than two billion people, and is highly prevalent in developing countries where cereals are staple foods. We have used genetic engineering to produce rice and wheat plants that are more effective at mining soil for Fe and transporting Fe to grain. These Fe “biofortified” plants contain significantly increased Fe concentrations in edible grain tissues, grow normally in multi-location field trials, and have high Fe bioavailability as indicated by the Caco-2 cell assay. The production of Fe biofortified cereal crops represents a sustainable intervention to address human Fe deficiency in developing countries at no additional cost to growers and food manufacturers.

Canola is the second largest oilseed crop worldwide used for human and animal feed. Canola cultivation has grown rapidly over the past five years owing to a high demand for canola oil and meal and is now Australia’s third largest broad-acre crop. Australia is the world’s second largest exporter of canola seed. With rising global demand for canola for food and non-food applications, its production is expected to increase by 40% by the year 2025. However, impending global climatic changes are predicted to hamper crop productivity. Salinity, drought and high temperatures are major environmental factors that limit agricultural yields. In this context, genetic improvement of crops for abiotic stress tolerance is vital to maintaining our food supply. Helicases, an important class of DEAD-box protein family are primarily known to unwind duplex nucleic acids to perform many housekeeping activities. These highly conserved enzymes play an essential role in several cellular processes including RNA metabolism and regulation of gene expressions. Here we report the development of abiotic stress tolerant canola lines by heterologous overexpression of a DNA/RNA helicase gene.
SYM-13-05
HIGH RESOLUTION ASSOCIATION MAPPING IMPLICATES NAS3 IN RICE ENDOSPERM ZN PHENOTYPE

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Approximately 30% of the global population suffers from dietary Zn deficiency, many of whom rely on rice to meet their daily caloric requirements. Biofortification represents an effective approach to improving nutritional and health outcomes, and for Zn, it is likely that sufficient natural variability exists to achieve these benefits through traditional plant breeding. The PRAY Indica diversity panel, consisting of 300 genotypes, was developed at IRRI for the GRSIP Global Rice Phenotyping Network (http://ricephenonetwork.irri.org) and grown for 4 seasons across 2 years in the Philippines. Grain was harvested at maturity and ionomic analysis performed by ICP-MS, quantifying 18 elements. In a separate study, a large subset of the panel was genotyped at 700k biallelic SNPs, and the resulting data was utilised in this work. Association mapping was performed using all quantified elements, allowing a broad analysis of genetic regions associated with ionomic phenotypes. Several QTLs for grain Zn were identified, the most significant and stable of which is located on chromosome 7. Candidate ionomic phenotypes. Several QTLs for grain Zn were identified, the most significant and stable of which is located on chromosome 7. Candidate gene analysis revealed the presence of the NAS3 gene within this QTL, whose product synthesises the metal chelator, nicotianamine (NA). Previous studies have identified NA-Zn complexes in the rice phloem, implicating NA in long distance micronutrient transport. This suggests a model for the effect of NAS3 on grain Zn phenotype in the current study.

SYM-14-01
NEW INSIGHTS INTO NEAT1 LONG NONCODING RNA AND PARASPECKLES

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Paraspeckles are mammalian subnuclear bodies that are built on the long noncoding RNA, NEAT1 (nuclear paraspeckle assembly transcript 1). Paraspeckles nucleate gene expression by the subnuclear sequestration, or sporing of specific transcription factors and the nuclear retention of some types of RNA. Within paraspeckles, the longer 23kb isoform of NEAT1, NEAT1_2, has a distinct organisation such that the 5-prime and 3-prime ends are found at the periphery and the middle is found at the centre. The shorter isoform of NEAT1, NEAT1_1 is dispensable for paraspeckle formation. I will present our latest results aimed at developing a coherent picture of how the different isoforms of NEAT1 lncRNA, the RNA binding proteins that bind them and the other transcripts modulated by these same proteins all act together to regulate gene expression in different disease contexts. Using CRISPR-Cas9 genome engineering we have created cell lines devoid of distinct NEAT1 isoforms and revealed potential paraspeckle independent functions for NEAT1_1. In addition, we use transient approaches to adjust NEAT1 isoform ratios and observe varying effects on NEAT1 binding proteins and cell viability. These insights are important for future efforts to therapeutically modulate NEAT1, particularly in the cancer context where NEAT1 was recently shown to be required for formation of distinct tumor types.

SYM-14-02
NOVEL CHROMATIN STATE CHANGES IN NEURAL DEVELOPMENT

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A key question in developmental biology is how cellular differentiation is controlled during development. Particular interest has focused upon changes in chromatin state, with transitions between Trithorax-group (TrxG) and Polycomb-group (PcG) states vital for the differentiation of ES cells to multipotent stem cells. Recently a number of other chromatin states have been shown to exist in cell culture, including a repressive “Black” chromatin state devoid of common chromatin marks. However, little is known as to the role of chromatin states during the development of complex organs such as the brain. In order to understand the role chromatin states play in neural development, we used the Targeted DamID system to profile chromatin states within the developing fruit fly brain. We obtained genome-wide binding profiles of five key chromatin proteins in three separate cell types -- neural stem cells (NSCs), immature neurons and mature neurons -- and we determined chromatin states through a Hidden Markov Model approach. We demonstrate that the majority of genes that are activated during neuronal differentiation are repressed by the Black chromatin state in NSCs. Furthermore, almost all key NSC genes are switched off via a transition to HP1-mediated repression. Interestingly, PcG-mediated repression does not play a significant role in regulating either of these transitions; instead, PcG chromatin specifically regulates lineage-specific transcription factors that control the spatial and temporal patterning of the brain. Combined, our data suggest that forms of chromatin other than canonical PcG/TrxG transitions take over key roles during neural development.

SYM-14-03
BHLH-PAS TRANSCRIPTION FACTORS: TARGET GENE SELECTION, SATIETY AND THE HOMEODOMAIN CONNECTION

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bHLH-PAS transcription factors are a family of developmental and rheostatic gene regulators that control central biological processes to maintain cellular and tissue homeostasis. These include the control of oxygen supply and utilization (Hypoxia Inducible Factors (HIF1α/HIF2α)), sensing and detoxification of environmental pollutants (Aryl hydrocarbon Receptor (AhR)), maintenance of satiety signaling and body weight (Single Minded 1 (SIM1)), balancing neuronal network activity (Neuronal PAS transcription factors (NPAS1/NPAS3/NPAS4)), and the coordination of circadian cycling (CLOCK/NPAS2/BMAL), bHLH-PAS transcription factors act as heterodimers (with ARNT/ARNT2/BMAL) to bind to similar and in some instances shared atypical E-BOX like DNA elements (NNCCTG) to illicit changes in gene expression. Given the degeneracy in the known DNA binding elements which this class of transcription factors bind, it is unclear how target gene selectivity is achieved. We used SELEX-seq to define the DNA binding sites of bHLH-PAS transcription factors and provide evidence that selectivity may be achieved through inherent DNA sequence selection and modified by response element methylation. Recently we showed that loss of function (LoF) variants in the satiety signaling bHLH-PAS transcription factor SIM1 are associated with severe obesity in humans. Using a patient derived SIM1.R171H point mutant mouse model, we demonstrate that SIM1 hypomorphs can drive monogenic, hyperphagic obesity. We also demonstrate that SIM1 can form a novel complex with the paired-homebox transcription factor Orthopedia (OTP) to alter reporter gene activation. Both Sim1 and Otp expression overlap within the satiety neuronal circuitry of the paraventricular nucleus (PVN) and LoF mutations in both Otp and Sim1 lead to obesity in mouse models. This suggests that in addition to Sim1 and Otp being essential for early development in the hypothalamus, the novel SIM1/OTP transcription factor complex may distinctly specify the expression of genes involved in satiety within the appetite circuits of the PVN.
SYM-14-04

EFFICIENT LEVELS OF MINOR CLASS SPlicing ARE REQUIRED DURING VERTEBRATE DEVELOPMENT AND FOR MULTIPLE FORMS OF CANCER

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A small sub-set (<0.5%) of introns in the vertebrate genome harbour distinctive 5’ and 3’ splice sites that require recognition by a unique spliceosome for their excision. We have shown that zebrafish and mice carrying loss-of-function mutations in a specific minor class spliceosome component, Rnpc3, die during development. Transcriptome analysis of rnpc3−/− larval (72hpf) demonstrates that minor class splicing is required for the proper expression of genes involved in transcription, splicing and nuclear export. Since these genes are essential for the growth and division of rapidly proliferating cells, we hypothesise that efficient minor class splicing will also be crucial for cancer cells. rnpc3−/− zebrafish and mice develop normally with no obvious phenotype. However, Rnpc3 heterozygosity results in a significantly reduced tumour burden in mouse models of lymphoma, leukaemia, lung and gastric cancer. We also obtained similar results with a zebrafish model of human hepatocellular carcinoma (HCC), whereby liver-specific expression of a doxycycline-inducible transgene (EGFP-Kras120) produces robust and reproducible levels of hepatocytic hyperplasia that can be accurately quantitated with 2-photon microscopy. Using EdU incorporation analysis we found that the reduced liver volume observed on a rnpc3−/− background was caused, at least in part, by a decrease in cell proliferation. Our results indicate that minor class splicing represents an attractive anti-cancer target with a therapeutic window that could be exploited clinically to restrict the growth of cancer cells without affecting normal tissues.

SYM-14-05

NOVEL CO-OPTION OF THE CARDIAC TRANSCRIPTION FACTOR NKK2.5 DURING DEVELOPMENT OF THE EMU WING

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A major issue in the field of Evolutionary Developmental Biology ( EvoDevo) is the relative contribution of changes in gene structure versus gene regulation in driving morphological diversity. The vertebrate embryonic limb bud is an ideal model system in which to explore these issues. Among birds, for example, the flightless raptors have highly divergent wing structure. The emu embryo has a vestigial wing, which develops from a greatly reduced bud during embryogenesis. Using a comparative genomics approach, we have identified a novel co-option of the cardiac transcription factor, Nkk2.5, to the early forelimb bud of the emu embryo, but not in ostrich, zebra finch or chicken, which have fully developed wings. Nkk2.5 is expressed in emu myogenic and non-myogenic limb precursors and mature muscle cells. Remarkably, mis-expression of Nkk2.5 in the chicken embryonic limb bud results in wing reductions comparable to those seen in the emu. We propose that Nkk2.5 functions to inhibit muscle growth and development in the emu wing. Changes in the regulation of Nkk2.5 have resulted in novel expression and function in the emu lineage, playing a role in the evolution of wing reduction.

SYM-15-01

YOU ARE WHAT YOU DO NOT EAT: BACTERIAL ADAPTATION TO NUTRITIONAL IMMUNITY

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During infection, Staphylococcus aureus and other pathogens must obtain all of their nutrients from the host. To combat invaders, vertebrates take advantage of this fact and restrict the availability of essential nutrients such as manganese and zinc. Currently, the adaptations that enable pathogens to overcome host-imposed manganese and zinc starvation remain largely unknown. Utilizing the manganese and zinc binding immune effector calprotectin and mice with defects in metal sequestration, we have begun to elucidate how S. aureus overcomes this defense known as nutritional immunity. Differing from most staphylococci, S. aureus possesses two superoxide dismutases. Our investigations have revealed that acquisition of the second superoxide dismutase, SodM, enables S. aureus to overcome nutritional immunity and cause infection. Differing from SodA, which all staphylococci have, our analysis revealed that SodM is capable of functioning with either manganese or iron. The cambialistic nature of SodM enables S. aureus to retain an antioxidant defense in the presence of calprotectin and better resist oxidative stress. While biochemical studies have previously suggested the existence of superoxide dismutases capable of using iron or manganese, the biological importance of cambialism has remained controversial. Cumulatively, these studies provide a mechanistic rationale for the acquisition of a second superoxide dismutase by S. aureus and demonstrate an important contribution of cambialistic superoxide dismutases to bacterial pathogenesis. Furthermore, they also suggest a new mechanism for resisting manganese starvation, namely populating manganese-utilizing enzymes with iron.

SYM-15-02

THE STRUCTURAL BIOLOGY OF COMPLEX IV ASSEMBLY

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The mitochondrial oxidative phosphorylation system generates the bulk of cellular ATP, fuelling the energy demands of most eukaryotes. Five multi-subunit protein complexes in the mitochondrial inner membrane, termed complexes I to V, comprise the oxidative phosphorylation system. Copper is an essential cofactor for the enzyme cytochrome c oxidase (CcO; Complex IV), which is the terminal oxidase of the mitochondrial respiratory chain and requires three copper ions for assembly and activity of the complex. The protein Coa6 is located in the intermembrane space of mitochondria and is required for CcO assembly and activity. Our recent work has shown that Coa6 binds Cu(I), however the mechanism of how Coa6 mediates Cu-delivery is unknown. Studies on a clinically-relevant mutation of the Coa6 protein, C59W, have proposed that the mutation acts to disrupt protein-protein interactions between Coa6 and its proposed protein partners with identified roles in CcO assembly, leading to dysfunctions in CcO incorporation into CcO. This presentation will describe the crystal structure of the native Coa6 and W59C mutant proteins and implications for the role of this protein in CcO assembly and function.
SYM-15-03
TARGETING ZINC: A PATHWAY TO IMPROVE COGNITION IN HEALTH AND DISEASE?
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One of the critical cell processes that becomes dysregulated with age and also in disease, and which participates both directly and indirectly in cognitive function, is metal homeostasis and the neurochemistry of metalloproteins. This is particularly true for zinc, in which 10–15% of brain zinc exists in a chelatable form primarily within synaptic vesicles at glutamatergic synapses, highlighting its potential importance in synaptic plasticity/cognition. Our studies have proven that alterations to this available zinc pool, via ablation of the synaptic zinc transporter (ZnT3), results in an age-dependent cognitive phenotype that is underscored by significant deficits in key proteins involved in synaptic plasticity (eg NMDA and AMPA receptors). Furthermore, the partial pharmacological restoration of zinc levels within key brain structures (using both ZnT3 KO and normal aged WT mice) is sufficient to normalise cognition in the whole animal, restore long-term potentiation in ex vivo brain slices and elevate key proteins involved in learning and memory. Taken together with other supporting data in the literature, this demonstrates a critical role for zinc in learning and memory which may be relevant to cognitive function, and which may also be a therapeutic target for improving functional outcomes in health and disease.

SYM-15-05
CONTINUOUS MASS SPECTROMETRY ASSAY FOR TOXIC PHOSPHOLIPASE FROM AUSTRALIAN SNAKE VENOMS
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Phospholipases A\textsubscript{2} (PLA\textsubscript{2}) are a common component of Australian snake venoms that can act as neurotoxins, myotoxins and/or have antiplatelet activities. Some of these enzymes possess the ability to hydrolyze sphingomyelin (SM) in plasma and induce toxicity. These enzymes cause neuronal dysfunction, muscle paralysis and hemorrhagic symptoms. Phospholipases A\textsubscript{2} have been shown to produce a variety of toxic effects in vivo, including paralysis, cardiovascular collapse, respiratory failure and death. However, there is a need for new methods to study phospholipase activity in a continuous fashion. The aim of this work was to develop a continuous assay using electrospray ionization mass spectrometry (ESI-MS) to measure the hydrolysis of lipids in liposomes, which act as analogues of cell membranes. Although, ESI-MS has previously been used to investigate the activities of PLA\textsubscript{2}, where the ratio of lipid:lysophosphatidyl:cholesterol was quantified for multiple lipid species, these methods require reaction quenching and analysis by liquid chromatography mass spectrometry (LC-MS). In this research we describe an ESI-MS assay that uses more physiologically relevant substrates, in a continuous assay to obtain valuable kinetic data for these toxins.

SYM-15-04
ZINC HOMEOSTASIS IN STREPTOCOCCUS PNEUMONIAE DURING DISEASE
Eijkelkamp B.A.1, Morey J.R.1, Pederick V.G.1, Cole N.2, Singh P.P.2, Hughes C.1, Pumpltent G.D.1, Begg S.L.1, Ong C.-L.Y.1, Paton J.C.1, McEwan A.1, Doble P.1 and Minnold G.S.1
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Acquisition of zinc by Streptococcus pneumoniae is essential for colonization and mediating disease. Zinc uptake in S. pneumoniae occurs via the ATP-binding cassette transporter AdcBC, and two zinc-binding proteins, AdcA and AdcAll. We have previously shown that AdcA and AdcAll act in a complementary manner during host colonization to facilitate a more efficient infection, but our analyses also revealed that only AdcAll is reliant upon the pneumococcal histidine triad (Pht) proteins. We have also shown a hierarchical importance of the S. pneumoniae histidine triad motifs present in PhtD, with the N-terminal histidine being the most significant and the C-terminal histidine triad the least in zinc acquisition. Upon exposure to potentially toxic zinc concentrations, the pneumococcous utilizes the zinc efflux protein CzcD to efficiently reduce intracellular zinc concentrations. To examine the concerted mode of action of all zinc homeostasis mechanisms during pneumococcal infection, we have established a murine zinc-deficiency model. These analyses have revealed that the bacteria elicit the redistribution of tissue zinc, although, this is highly niche-specific and dependent on zinc availability. Interestingly, this redistribution directly targets S. pneumoniae by dysregulating its intracellular metal ion homeostasis, thereby providing a basis for the antimicrobial role of host zinc during infection. Overall, we propose that zinc supplementation may be an effective disease prevention strategy in groups at increased risk of contracting S. pneumoniae infections.

SYM-15-06
THE USE OF BIG DATA FOR THE STUDY OF SOMATIC MUTATION ACCUMULATION AND REPAIR WITHIN CIS-REGULATORY REGIONS OF CANCER GENOMES
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Mutations that directly alter protein function have been extensively studied in cancer. However, in recent years, it has become feasible to examine the cancer-causing role of mutations within the remaining 98% of the genome which is non-coding. Here I will present our use of big data for the study of cis-regulatory somatic mutations in cancer genomes. We analysed somatic mutations from over 1,000 cancer genomes across 14 cancer types, finding many cancers to exhibit increased somatic mutation density at promoter elements. By analysing genome-wide maps of nucleotide excision repair (NER), we discovered that NER activity is reduced within the DNAs of hypersensitive centre of gene promoters, inversely mirroring the increased mutation density. Our analyses uncovered the presence of a previously unknown mechanism by which we implicate localised differential DNA repair caused by the binding of transcription factors in producing somatic mutation hotspots in gene promoters in many cancer types. We extended our analyses further by studying mutation accumulation in skin cancers at the binding sites of the protein CTCF. In doing so, we observed a unique and asymmetric mutation pattern within CTCF motifs. Examining NER, CTCF binding and replication timing datasets, we showed this mutation pattern to be attributable to ultraviolet irradiation and differential NER specifically across individual nucleotides within the CTCF motif. To demonstrate that CTCF binding site mutations can be functional, we performed CTCF CHIP-seq in a melanoma cell-line to show allele-specific reduction of CTCF binding to mutant alleles. The frequency and potential functional impact of cis-regulatory somatic mutations highlights the need to consider their impact on cellular phenotype in cancer genomes.
### SYM-16-02

**CHALLENGES OF SINGLE-CELL RNA-SEQ DATA ANALYSIS**

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Single-cell RNA sequencing (scRNA-seq) enables researchers to interrogate the genome-wide expression profile of tens of thousands of individual cells. This has opened up new opportunities in all areas of biological and biomedical research. Yet, there are a number of analytical challenges associated with analysing scRNA-seq data. In this talk, we will discuss two major challenges - scalability and dropouts - and our two recently published bioinformatics tools that deal with these challenges - Falco and CIDR. Falco is a cloud-based framework to enable parallelisation of existing RNA-seq processing pipelines using big data technologies of Apache Hadoop and Apache Spark for enabling parallelisation of existing RNA-seq processing pipelines. CIDR is a dimensionality reduction and clustering tool that alleviates the impact of dropouts using a novel ‘implicit imputation’ approach.

### SYM-16-03

**HLA AND KIR TYPING IN LARGE COHORTS: INSIGHTS FOR STUDIES OF DISEASE**

Leslie S.  
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Human leukocyte antigen (HLA) genes are immune-system genes that are of major biological and clinical interest. They are located in the major histocompatibility complex (MHC) on human chromosome 6, and play a key role in the recognition of self and non-self. The MHC has evolved to a high level of diversity, almost certainly in response to infectious diseases, but most likely at the cost of increased susceptibility to immune-mediated diseases. The region stands out due to this diversity and its complexity. HLA alleles are determinants of transplant compatibility, and have been associated with many conditions including autoimmune diseases, communicable diseases, cancer and adverse drug reactions. Also, of key interest are genes encoding the killer-cell immunoglobulin-like receptors (KIRs), which have been and are hypothesised roles in autoimmune diseases, resistance to viruses, reproductive conditions and cancer. They are similarly polymorphic to the HLA genes, again probably in response to pathogens. Due to this extreme diversity HLA and KIR alleles are expensive to type at high resolution using lab-based methods, meaning they are often neglected in studies of genetic association to disease/other phenotypes. A major advance was the development of statistical methods (HLA/KIR imputation) that use the correlation structure between HLA/KIR genes and nearby SNPs to type unknown HLA/KIR alleles. HLA/KIR imputation is high-throughput, accurate and inexpensive. It has enabled large-scale studies of genetic variation in the MHC, and significantly advanced the understanding of many diseases including the first discovered genetic interactions associated with human disease. This talk will give the key ideas behind HLA/KIR imputation methods. It will then show recent work using HLA typing in very large samples, demonstrating the insights obtainable using HLA/KIR imputation in human disease genetics research.

### SYM-16-04

**DETECTION AND QUANTIFICATION OF INBREEDING DEPRESSION FOR COMPLEX TRAITS FROM SNP DATA**

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Mating between close relatives has detrimental consequences on the survival and fertility of resulting offspring. This reduction in fitness, also termed as inbreeding depression (ID), can be estimated from SNP data in unrelated individuals using a number of measures of inbreeding. Our study addresses two key questions. How accurate are the different methods to estimate ID? How and why should investigators choose among the multiple inbreeding measures to detect and quantify ID? Here, we compare the behaviours of ID estimates from three commonly used SNP-based measures of inbreeding (F_R, runs of homozygosity, F_ORS, excess homozygosity and F_UNI, correlation between uniting gametic phase under a classical quantitative genetics framework. We derive mathematically and illustrated through simulations that ID can be estimated using F_UNI is unbiased in a wide range of situations, while the commonly used measure F_IN is biased. We demonstrate that heterogeneity in linkage disequilibrium (LD) between causal variants and SNPs creates biases in ID estimates and we developed a method to correct these biases using LD and minor allele frequency stratification (LDMS). Using that method, we quantified ID in 25 quantitative traits measured in ~140,000 participants of the UK Biobank. We confirmed previously published associations between inbreeding and height, lung function, cognitive function and fertility related traits and found new evidence (ID between -2.3 and -5.2 phenotypic SD for complete inbreeding; p<0.002) of ID for handgrip strength, waist/hip ratio and visual and auditory acuity. Our results illustrate how choosing a less variable measure of inbreeding combined with LDMS stratification can improve both detection and quantification of ID using SNP data.

### SYM-16-05

**WILD WINE: METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES DURING WINE FERMENTATION**

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Wine is a complex beverage, comprising thousands of metabolites that are produced by yeasts and bacteria acting on grape must. To ensure a robust and reliable fermentation, most wines are produced by inoculating grapes with specific commercial strains of the wine yeast Saccharomyces cerevisiae. However, there is a growing trend back to the historical practice of performing uninoculated or ‘wild’ fermentations, in which the multitude of different species of yeasts and bacteria that are naturally associated with the grapes or winery, are used. Wild ferments show a far more complex progression of microbial species than inoculated wines and, accordingly, a more complex taste and aroma profile. As such, differences in these resident microflora between vineyards and wineries are therefore thought to have a key role in defining regional expression of wine characteristics. In order to map the microflora of spontaneous fermentation, genomic and metagenomic techniques are being used to monitor the progression of microbial species in hundreds of wild fermentations from across the major winemaking areas of Australia. Notable differences between regions, vineyards and wineries were apparent and these can be broadly defined by the resulting microbial composition of the wild ferments.

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SYM-17-01
GENOME-WIDE CRISPR SCREENS TO IDENTIFY MECHANISMS OF CANCER THERAPY RESISTANCE

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Checkpoint blockade inhibitors have been successfully used in the clinic to treat a variety of cancer types. However, resistance to immunotherapy frequently occurs when the tumour acquires the capacity to evade the immune system. In order to improve current immunotherapies it is imperative to improve our understanding of tumour-immune escape mechanisms. By transducing tumour cells with genome-wide and targeted CRISPR gRNA libraries we have successfully performed in vitro and in vivo CRISPR screens to identify mechanisms of tumour immune escape from CD8+ T-cells. Our results identify loss of pro-inflammatory cytokine signaling and antigen presentation pathways as the major immune escape mechanisms. Moreover, our genome-wide approach also identifies novel regulators of these pathways. This comprehensive identification of tumour immune-escape mechanisms can be used to improve current therapies and aid in the development of early biomarkers of immunotherapy resistance.

SYM-17-02
TARGETING METABOLIC ADAPTATION IN BRAFV600E MELANOMA


Cancer cells rewire their metabolism in response to cellular stresses. In aerobic conditions normal cells metabolize glucose by oxidative metabolism, while cancer cells metabolize glucose to lactate using aerobic glycolysis. In the setting of BRAFV600E melanoma, sensitivity to the BRAF inhibitor Vemurafenib (Vem) correlates with glycolytic response, and oxidative metabolism contributes to drug tolerance and acquired resistance. To understand BRAFV600E-driven metabolism and responses to Vem, we performed a genome wide glycolysis screen in BRAFV600E melanoma cells, in the presence or absence of Vem. This approach uncovered RNA metabolism and translation pathways as major nodes in the BRAFV600E glycolysis network, including the RNA binding protein kinase UHMK1. Depletion of UHMK1 enhanced Vem sensitivity, synergistically suppressing glycolysis, proliferation, and viability, while analysis of oxidative metabolism revealed reduced spare respiratory capacity, glutamine dependency and ATP production. Analysis of mRNA translation using polysome profiling demonstrated selective translation of metabolic enzymes in cells adapting to BRAF inhibition, despite global inhibition of protein synthesis. Importantly, this adaptive translation program was partially overcome by depletion of UHMK1. Our data provide evidence of a therapy induced translational mechanism regulating adaptive metabolic responses in melanoma. We propose that UHMK1 represents an attractive therapeutic target to improve efficacy of BRAF inhibitors by preventing metabolic adaptation in BRAFV600E melanoma.

SYM-17-03
ROCK-EDUCATION OF MAMMARY TUMOUR FIBROBLASTS ENHANCES THEIR MALIGNANT PHENOTYPE AND CREATES A TUMOUR-PERMISSIVE MICROENVIRONMENT

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Breast cancer is the most commonly diagnosed cancer in females worldwide, and an unmet clinical need exists for novel approaches to target cancers refractory to current treatments. Having shown that Rho-associated kinase (ROCK) is hyper-activated in most breast cancers, we sought to determine whether ROCK activity has a causal role in promoting tumourigenesis. We have discovered that conditional hyper-activation of ROCK in a mouse model of breast cancer (PyMT) significantly enhances mammary tumour burden compared to that of mice expressing a kinase-dead (KD) version of ROCK. Similarly, blockade of ROCK by administration of the pharmacologic inhibitor Fasudil significantly delays mammary tumour onset and reduces tumour burden. Interestingly, hyper-activation of ROCK in mammary tumours results in an increased number of cancer-associated fibroblasts (CAFs) compared to that observed in KD mammary tumours. CAFs from PyMT-ROCK tumours, or those exposed to culture medium conditioned by PyMT-ROCK tumour cells, are highly invasive and strongly promote tumour growth from cancer cells upon transplantation when compared to CAFs from PyMT-KD tumours or those exposed to culture medium conditioned by PyMT-KD tumour cells. Our findings strongly suggest that ROCK regulates breast cancer progression by influencing the cancer-promoting properties of CAFs via fibroblast-educating paracrine signalling mediators. In cancer cells, the ROCK pathway is thought to be activated in response to changes in mechanical tension and biochemical signals from the tumour microenvironment. Indeed, we have found that mimicking the early mammary cancer environment of enhanced compressive stress rapidly activates the Rho-ROCK pathway in cells and tissues. As this pathway in tumours regulates the key mechanical properties of the tumour microenvironment, we propose that ROCK promotes cancer progression via a mechanoregulatory feed-forward mechanism. Using a number of cutting-edge techniques, we are now working on identifying the signalling mediators secreted from breast cancer cells upon ROCK activation and the effects they have on CAFs in order to develop new therapeutic strategies to target and impede the tumour-promoting functions of ROCK-mediated fibroblasts to normalise the tumour microenvironment.

SYM-17-04
SYSTEMS APPROACHES TO UNDERSTANDING THE ASSEMBLY OF MITOCHONDRIAL MACHINES

Stroud D.A., Formosa L.E., Lake N.J., Dibley M.G., Compton A.G., Thorburn D.R. and Ryan M.T. Department of Biochemistry and Molecular Biology, Monash Biomedicine Discovery Institute, Monash University, Melbourne, Victoria, Australia. ‘Munditch Childrens Research Institute, Royal Children’s Hospital, Melbourne, Victoria, Australia.

SYM-17-05
THE FUNCTIONAL INVESTIGATION OF DOCK1 CIRCULAR RNA

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Circular RNAs (circRNAs) are covalent circles of single strand RNA that arise from non-canonical, back-splicing of pre-mRNAs and non-coding RNAs. Our previous research showed that some circRNAs are specifically regulated in their abundance in epithelial to mesenchymal transition (EMT), which indicates they have functions associated with EMT and cancer progression. Within these circRNAs we are particularly interested in Dock1 circRNA which consists of 26 exons from Dock1 gene and spans more than 150kb over the human genome. Dock1 circRNA was strongly downregulated in EMT, despite the cognate mRNA being unchanged. We have found that Dock1 circRNA is widespread across different human tissues indicating it is likely to have important roles. We have identified an RNA-binding protein that regulates the production of Dock1 circRNA and are investigating the mechanism of this regulation. We are also investigating the function of the Dock1 circRNA by examining the effects of knockdown and overexpression of this circRNA on the properties of mammary epithelial and mesenchymal cells, respectively. Our study indicates Dock1 circRNA may have important roles in epithelial to mesenchymal transition.

SYM-18-01
EVOLUTION OF THE TOR FIELD

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TOR (Target of Rapamycin) was discovered in 1991. This highly conserved kinase and central controller of cell growth has since drawn the interest of basic scientists, medical researchers, and members of the pharmaceutical industry. The study of TOR has in turn grown into a large, complex field that is overwhelming and thus inaccessible to many. I will present a largely historical overview of the TOR (mTOR in mammals) field that will be aimed at specialists and non-specialists alike.

SYM-18-02
TARGETING THE RIBOSOME TO TREAT PI3K/MYC-DEPENDENT CANCER

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Many human cancers are characterized by deregulated growth factor and nutrient-sensing signaling through the PI3K/RAS/MYC network. Elevated signaling through this network drives increased rates of ribosome biogenesis and mRNA translation. Dock1 circRNA may have important roles. We have identified an RNA-binding protein that regulates the production of Dock1 circRNA and are investigating the mechanism of this regulation. We are also investigating the function of the Dock1 circRNA by examining the effects of knockdown and overexpression of this circRNA on the properties of mammary epithelial and mesenchymal cells, respectively. Our study indicates Dock1 circRNA may have important roles in epithelial to mesenchymal transition.

SYM-18-03
DETAILED FUNCTIONAL STUDIES OF TWO DIFFERENT FGFR2 MUTATIONS AND ANALYSES OF ACQUIRED RESISTANCE MODELS REVEAL NOVEL INSIGHTS INTO FGFR SIGNALING


Germline mutations at different positions along FGFR2 activate the receptor through different mechanisms and give rise to congenital syndromes with different phenotypes and severity. Some mutations, but not others, occur somatically in endometrial cancer and as a whole FGFR2 mutations are associated with poorer prognoses in Endo Ca. Given the genotype/phenotype correlations giving rise to different germline syndromes it is reasonable to assume that not all activating FGFR2 mutations are equal. We have transduced wildtype and two different FGFR2 mutations into cells and show that while they both result in golgi changes and loss of polarity, they result in difference in downstream signaling and different migration and invasion phenotypes. In addition to studying how FGFR2 mutations drive EC tumorigenesis we are also investigating both intrinsic and acquired resistance to FGFR inhibition in cancer cell lines carrying FGFR2 mutations. Through this we have identified a new mechanism of acquired resistance where gene mutation/loss not only activates the downstream MAPK pathway but also reduces expression of FGFR2 itself. Reinroduction studies reduce MAPK activation, increase FGFR2 expression and re-sensitise the cells to FGFR inhibition thereby implicating a new protein in the negative regulation of FGFR signaling. A mutation in this gene has been seen in a cholangiocarcinoma patient with an FGFR2 fusion that was initially sensitive to BGB398 but later acquired resistance supporting this pathway in other cancer types.
TESTIS DETERMINATION REQUIRES A SPECIFIC FGFR2 ISOFORM TO REPRESSION FOXL2

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Male sex determination in mammals relies on SRY-mediated up-regulation of SOX9 expression in XY gonads, whereas WNT/RSPO signalling and FOXL2 drive female sex determination in XX gonads. FGF9-signalling ensures sustained SOX9 expression through repression of one of the ovarian pathways (WNT signalling), while potential FGF-mediated repression of the FOXL2 pathway has not been explored. Previously, we demonstrated that FGFR2 is the receptor for FGF9 in the XY gonad, however a requirement for a specific isoform (FGFR2b or FGFR2c) was uncertain. Here, we show that the FGFR2c isoform is required for FGF9-mediated male sex determination. At 15.5dpc, XY Fgfr2c-/- mice (C57BL/6J genetic background) displayed complete male-to-female sex reversal. The impairment of pro-male molecular signalling in the gonad was evident as early as 11.5dpc by a reduced number of SOX9-positive cells. Subsequently at 12.5-13.5dpc, SOX9-positive cells were restricted to the posterior gonadal pole, and later these were replaced by FOXL2-expressing cells which emerged initially at the anterior pole then progressively populated the entire gonad. The loss of FOXL2 in XY Fgfr2c/-Foxl2 double mutants led to partial or complete rescue of gonadal sex reversal in XY Fgfr2c-/- mice. Our data together with previous findings suggest that testis determination involves FGFR2c-mediated repression of both the WNT4- and FOXL2-driven ovarian determining pathways.

ELEVATED CANONICAL WNT SIGNALLING DISRUPTS DEVELOPMENT OF THE EMBRYONIC MIDLINE AND MAY UNDERLIE CASES OF ZIC3-ASSOCIATED HETEROTAXY

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Heterotaxy is a congenital abnormality where the internal thoraco-abdominal organs demonstrate abnormal arrangement across the left-right (L-R) axis of the body. It can affect the development of the heart, liver, lungs, intestines, and spleen. The L-R embryonic axis is established early in embryogenesis when unidirectional signals emanate from a specialised structure at the embryonic midline, called the node, to initiate distinct molecular pathways on the left and right sides of the developing embryo. The gene most commonly mutated in human cases of Heterotaxy is the X-linked ZIC3, but the mechanism by which the ZIC3 transcription factor prevents Heterotaxy remains unknown. A genetic screen for mutations that affect murine embryogenesis identified a novel null allele of Zic3, called katun (Ka). The mutant embryos exhibit Heterotaxy and also incompletely penetrant, partial (posterior) axis duplications and anterior truncation. These latter two phenotypes are redolent of elevated canonical Wnt signalling and analysis of Ka embryos reveals ectopic expression of direct targets of Wnt/β-catenin mediated transcription in mutant embryos. ZIC3 is a member of the Zic family of transcriptional regulators and previous work has shown that ZIC proteins can inhibit Wnt/β-catenin mediated transcription when overexpressed in cell lines. This raises the possibility that dysregulated Wnt signalling may contribute to Heterotaxy. We have investigated this notion by analysis of the murine batface (Bfc) gain-of-function allele of β-catenin that results in elevated Wnt/β-catenin signalling. We find this strain exhibits incompletely penetrant defects of L-R axis formation and synergises with the Zic3 Ka allele to produce an increased incidence of L-R axis defects. In both the Ka and Bfc strains, the node of homozygous embryos is misshapen and contains patches of non-ciliated cells that express endoderm genes. Moreover we find that human ZIC3-Heterotaxy associated mutations encode proteins that are defective in their ability to inhibit Wnt/β-catenin mediated transcription. Overall this provides strong evidence that Wnt dysregulation may underlie cases of ZIC3-associated Heterotaxy.
SYM-19-01
PASSIVE KNOWLEDGE TRANSFER IS NOT A THING: GETTING ACTIVE LEARNING INTO LARGE CLASSES
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The evidence is overwhelming that students learn better over the long-term if their learning process is active rather than passive. Students should be actively considering, applying and questioning the information that is presented in class, but this needs to be built into the learning activities to prevent passivity. It may seem to be a significant challenge to include active learning into our typical Australian university large lectures and tutorials, but there are some simple methods that work. In fact, active learning is also required to break the serious scientific misconceptions that students often bring into the class. These misconceptions can come from naïve understandings of the world based on subjective experience, or from the media, or even from how they have been trialled with individual misconceptions, but the next great challenge is to equip students with an ability to identify, query and correct their own misconceptions. This will require self-regulation, which is the ability to monitor one’s own learning.

SYM-19-03
FOSTERING EMPLOYABILITY SKILL DEVELOPMENT OF UNDERGRADUATE BIOCHEMISTRY STUDENTS THROUGH INCORPORATION OF E-NOTEBOOKS IN TEACHING
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The development of students employability skills is arguably the core business of todays universities. For students entering science research careers, they enter at a time when many funding agencies have a requirement for data to be collected and stored using reliable and quality assured data management processes. Additionally, as researchers we are collaborating more widely, with the demise of the ‘fake’ facts. Educators must sift through vast amounts of information to identify key content to ensure they present a current understanding of core principles for which they must ensure students have the skills to access, process and apply in a discipline appropriate manner. Students must contend with differentiating amongst a glut of easily accessible real and ‘fake’ facts. Educators must sift through vast amounts of information and communicate effectively. Students must learn to distinguish amongst a glut of ever increasing, easily accessible real and ‘fake’ facts. Educators must sift through vast amounts of information to identify key content to ensure they present a current understanding of core principles for which they must ensure students have the skills to access, process and apply in a discipline appropriate manner. Students must contend with differentiating amongst a glut of easily accessible “facts” and “fictions” to support their own understanding of core principles of which they often have little to no understanding.

SYM-19-02
INTERDISCIPLINARY APPROACH TO PROMOTE LIFELONG LEARNING OF SCIENTIFIC COMMUNICATION SKILLS
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In order to acquire lifelong scientific communication skills during tertiary studies, it is important that students are not only taught the relevant skill set, but that they also learn to apply these skills in the context of their chosen discipline. Unfortunately, variation in the teaching of scientific writing between disciplines (and sometimes individual subjects) at the undergraduate level can undermine the development of these important skills. Often, undergraduate students fail to recognize that the skills are applicable to the “real world”, but rather a task required to achieve a good grade in a particular subject. This contrasts with the intended learning outcomes of developing versatile, adaptable, lifelong scientific writing skills that we, as educators, aim to teach our students. If approached with the wrong learning attitude or beliefs, these skills will soon be forgotten after graduation. Academics from all six disciplines in the School of Biomedical Sciences at the University of Melbourne have collaborated in an interdisciplinary project designed to harmonise the teaching of scientific writing skills across the school. Drawing upon scientific writing themes common to each discipline (Biochemistry and Molecular Biology, Pathology, Physiology, Pharmacology and Therapeutics, Anatomy and Neuroscience and Microbiology & Immunology), the project aims to deliver consistent messages to students about the importance, purpose and conventions of scientific writing across all biomedical fields and to foster lifelong learning and skill acquisition.

SYM-19-04
FIRST YEAR AUTHENTIC RESEARCH: A PATHWAY TO GRADUATE ATTRIBUTES AND EMPLOYABILITY?
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Twenty-first century tertiary students and educators are faced with the challenge of deciphering ever increasing, easily accessible real and ‘fake’ facts. Educators must sift through vast amounts of information to identify key content to ensure they present a current understanding of core principles for which they must ensure students have the skills to access, process and apply in a discipline appropriate manner. Students must contend with differentiating amongst a glut of easily accessible “facts” and “fictions” to support their own understanding of core principles of which they often have little to no understanding.

As twenty first century educators, how do we develop the myriad of skills, including information processing and critical thinking, which students require to succeed academically and professionally? Engaging undergraduate students in authentic research has been included in the list of high-impact educational practices by George Kuh in “High-Impact Educational Practices: What They Are, Who Has Access to Them, and Why They Matter”. Undergraduate research activities allow educators to ‘reshape’ their curriculum to connect ‘key concepts and questions’ for early and active involvement of students’ in research based skills. Undertaking high quality research requires students to ask questions, gather and critique information, generate and organise new information, and/or new ways of considering existing information and communicate and apply the information in an appropriate context specific format. Involving students in these educational experiences from the beginning of their university tenure provides the students a solid learning platform to engage in their continuing education. Educators are afforded the opportunity to shape course curriculum to explicitly develop and assess the key graduate attributes which have been defined by universities to highlight the employability of the students matriculating from their institution. In this presentation we will review our approach and experience of implementing and scaffolding authentic undergraduate research across two first year undergraduate topics.
ACCLIMATION OF C4 PHOTOSYNTHESIS TO LOW LIGHT
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The CO2 concentrating mechanism (CCM) in C4 photosynthesis and the inevitable CO2 leakage out of the bundle sheath require additional energy cost which may limit the productivity of C4 plants in low-light environments. Our understanding of how the various biochemical subtypes of C4 photosynthesis respond and acclimate to low light remains unclear. Consequently, C4 grasses belonging to the NADP-ME, NAD-ME and PEP-CK subtypes were grown under 100% (control) environments. Our previous research in Arabidopsis thaliana and the degradation of mitochondrial protein during oxidative stress encompass the regulation of gene expression encoding mitochondrial proteins (both mitochondrial and nuclear encoded), protein import and the degradation of mitochondrial protein during oxidative stress or development. Our previous research in Arabidopsis thaliana has revealed that TIM17:23 and respiratory Complex I share at least one subunit, a pore forming member of the PE protein and Amino acid Transporter family (PRAT) Tim23-2. Furthermore, an increase in TIM17:23 led to a dramatic reduction in complex I, and vice versa, where the reduction of the amount of respiratory complex I via a variety of independent genetic approaches, led to a dramatic increase in the TIM17:23 translocase. These findings indicating a link between Tim23-2 and Complex I revealed that by changing the abundance of Tim23-2, mitochondrial biogenesis can be altered and provides an experimental approach to uncover the regulators of mitochondrial biogenesis and examine mitochondrial biogenesis in a dynamic model.
SYM-20-04

SHOOT-ROOT CARBON ALLOCATION AND SUGAR SIGNALING

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Roots and shoots are physically distant but functionally interdependent. The growth and development of these two organs systems compete for energy and nutrient resource, and yet, they keep a dynamic balance with each other. The success of such a relationship depends on efficient root-shoot communication. Aside from the well-known signaling processes mediated by hormones such as auxin and cytokinin, photosynthetic sugars have recently been shown to act as a rapid signal in coordinating root and shoot development in response to endogenous and exogenous clues, in parallel to their function as carbon and energy resources for biomass production. Novel findings from studies on vascular fluids have provided molecular insights into the role of sugars in long-distance communications between shoot and root. We will summarize here the current understanding of the possible routes for long-distance sugar transport within the plant body, and the impacts of sugar allocation and signaling on balancing root-shoot development. The shoot-root carbon-nitrogen allocation will be discussed as an example to illustrate the communication between the two organs through multi-layer root-shoot-root signaling circuits, comprising sugar, nitrogen, cytokinin, auxin and vascular small peptide signals.

SYM-21-01

DISSECTING THE REGULATION OF NEPHRON PROGENITOR IDENTITY, SELF-RENEWAL AND COMMITMENT

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Low nephron number is a major risk factor for chronic kidney disease and is associated with environmental and genetic factors that impair kidney development. Despite this, we are yet to understand how nephron number is regulated. Nephrons arise from a nephron progenitor population located at the periphery of the developing organ. While lineage tracing shows that NP cells give rise to all epithelial cell types in the nephrons, this population also drives branching of the cells within the underlying ureteric tip. Nephron progenitor (NP) signals drive ureteric tip branching. Reciprocal signals from the tip promote NP self-renewal and also trigger a portion of these cells to differentiate into nephrons. Thus, the balance of NP self-renewal and differentiation determines final nephron number and is critical for optimal kidney development. We have developed high resolution multiscale imaging approaches to quantitatively and temporally evaluate kidney development in the mouse. Our work has challenged the current view of NP regulation, identifying new cellular heterogeneity, novel pathways contributing to NP turnover, and complex competing signals controlling the migration and commitment of NP cells. This new data challenges our current view of nephron commitment. Current work now focuses on dissecting NP regulation using high throughput single cell sequencing to interrogate pathways involved in nephron progenitor expansion and commitment, and to evaluate the cellular composition of human kidney organoids. By further increasing our understanding of nephron progenitor identity, turnover and commitment, we may identify strategies to increase renal capacity in vivo, and manipulate nephron progenitors in culture for tissue engineering and drug-screening applications.

SYM-20-05

THE ROLE OF THE CHLOROPLAST SIGNAL 3’PHOSPHOADEENOSINE 5’PHOSPHATE, IN STOMATAL CLOSURE

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When plant experiences drought stress, a number of molecular and physiological processes are activated. This includes the production of a chloroplast-to-nucleus stress signal, 3’phosphoadenosine 5’phosphate (PAP), which up-regulates transcription of stress-responsive genes in Arabidopsis thaliana leaves. However, the functional role of chloroplasts and specifically PAP-mediated signaling remains enigmatic in guard cells, which are the gatekeepers of water loss during drought. We show that exogenous application of PAP leads to rapid stomatal closure in both epidermal peels and intact leaves to a similar extent as the guard cell regulatory hormone abscisic acid (ABA). Significantly, genetic analyses and biochemical manipulation experiments indicate that PAP signaling can supplement the canonical ABA-mediated signaling pathway in guard cells, including inducing a reactive oxygen species burst that coincides with stomatal closure. PAP signaling in guard cells can act at least in part by up-regulating a number of novel calcium-dependent protein kinases that were previously not known to function in guard cells. Coupled with affinity chromatography to identify potential protein interactors, we have found numerous proteins and complexes that interact with PAP, providing potential mechanisms and components for PAP to affect sensitive closure pathway. This work presents evidence of the chloroplast signal PAP affecting numerous signaling components in stomatal closure in addition to its role as a retrograde signal. These results indicate PAP has a functional role as a molecular signal guiding water stress tolerance via guard cells.

SYM-21-02

FROM SERVANT TO MASTER: THE RIBOSOMES INSTRUCTIVE ROLE IN CELL FATE DETERMINATION

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Much focus has been given to transcriptional control of gene expression as the key determinant of stem cell fate and tissue development, with the assumption that translation of the resulting mRNA species is a passive event. Intuitively, impaired ribosome function should (and frequently does) result in reduced tissue growth and stunted development. However, studies from both Drosophila, zebrafish and a range of human disease states suggest that a reduced level of the protein building blocks of the ribosome (the ribosomal proteins, RPs) can, counter-intuitively, drive tissue overgrowth. We have demonstrated that tissue specific depletion of certain RPs (RPs19 and s24) in the Drosophila hematopoietic organ (the lymph gland) disrupts stem cell maintenance and, paradoxically, drives tissue overgrowth. Interestingly, knockdown of RPs19 and RPs24 differentially altered stem and progenitor cell differentiation, which suggests ribosomes might actively determine cell fate by modulating the classes of transcripts translated. Mass Spec data from the RP deficient lymph glands revealed increased protein abundance for several factors previously implicated in driving developmental programs of growth and differentiation, including master transcriptional regulators and chromatin remodeling machinery. As RNA-Seq revealed mRNA transcript levels for these factors were unchanged, we hypothesise that their defective translation underlies the stem cell fate defects and tissue overgrowth. We are currently testing whether increased abundance of our putative translational targets is necessary for overgrowth associated with RP depletion.
SYM-21-03

NAD DEFICIENCY, CONGENITAL MALFORMATIONS AND NIACIN SUPPLEMENTATION

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Congenital malformations can manifest as combinations of phenotypes that co-occur more often than expected by chance. In many such cases it has proved difficult to identify a genetic cause. We sought the genetic causes of cardiac, vertebral and renal defects, amongst others, in four unrelated patients. Genomic sequencing was used to identify potentially pathogenic gene variants in four families. The variants were functionally tested using in vitro enzyme activity assays, and by quantifying metabolites in patient plasma. We engineered mouse models using CRISPR/Cas9. Variants were identified in 3-hydroxyanthranilate 3,4-dioxygenase (HAAO) and kynureninase (KYNU) genes encoding kynurenine pathway enzymes. Three patients carried homozygous variants predicting loss-of-function changes in the HAAO or KYNU proteins (HAAO p.D162*; HAAO p.W186*; or KYNU p.V57Efs*21). Another patient carried heterozygous KYNU variants (p.Y156* and p.F349Kfs*4). The mutant enzymes had greatly reduced activity in vitro. Nicotinamide adenine dinucleotide (NAD) is synthesized de novo from tryptophan via the kynurenine pathway. The patients had reduced circulating NAD levels. Haao or Kyru null mouse embryos developed similar defects to the patients due to NAD deficiency. In null mice, averting NAD deficiency during gestation prevented defects. In conclusions, disruption of NAD synthesis causes a deficiency and congenital malformations in humans and mice. Niacin supplementation during gestation prevented the malformations in mice.

SYM-21-04

PERIPHERAL AND CENTRAL NERVOUS SYSTEM DEFECTS IN A MOUSE MODEL OF GOLDBERG-SHPRINTZEN MEGACOLON SYNDROME

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Goldberg-Shprintzen megacolon syndrome (GOSH) is a rare congenital disorder characterised by severe intellectual disability, craniofacial dysmorphism, microcephaly, and Hirschsprung disease. GOSH is caused by mutations in the gene encoding Kinesin Binding Protein (KBP; Kiaa1279), which has a role in axonal outgrowth and maintenance. We aimed to develop a mouse model of GOSHS and to examine the role of KBP in the development of the extrinsic and intrinsic innervation of the gut, and the brain. Kbp knockout mice were generated by CRISPR/Cas9 genome editing, targeting exon 1 of murine Kiaa1279. Although GOSH mice have Hirschsprung disease, neurons were present along the entire large intestine of newborn Kbp-/- mice. However, we found a significant delay in enteric neural crest cell migration in E12.5 Kbp-/- embryos. The projections of vagal fibres into the stomach were significantly reduced in Kbp-/- mice, as was the vagal innervation to the pancreas and lungs. The development of the sympathetic innervation of the intestine was also delayed in Kbp-/- mice. Newborn Kbp-/- mice were cyanotic, had breathing difficulties and died within several hours of birth. There were no obvious defects in the development of the phrenic nerve projections to the diaphragm or in the respiratory centres in the brainstem of newborn Kbp-/- mice, however some of the white matter tracts in the brain were reduced in size. The olfactory bulbs were significantly smaller in Kbp-/- mice and lacked normal organisation. The observed defects indicate an important role for KBP in axonal projection in the peripheral and central nervous system and etiology of GOSH.

SYM-21-05

GRAINYHEAD PROTEINS REGULATE INTESTINAL STEM CELL FUNCTION

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Snail family transcription factors are required for maintenance of intestinal epithelial stem cell pools and for regulation of daughter cell lineage choice in both mammals and Drosophila. This family of proteins and other mesenchymal inducers have been shown to be associated with stem cell potential in a variety of epithelial tissues but it remains curious that these stem cells maintain an epithelial identity. We considered that other proteins may also be required to maintain intestinal epithelial stem cell (ISC) identity in the presence of Snail proteins. We have identified that loss of Grainyhead results in loss of ISCs and a reduction in the size of clones that originate from ISCs. Grainyhead is expressed at very low levels in the intestine. Alternative splicing of the grainyhead gene produces 8 mRNA transcripts, which is in contrast to Kemp eliminase, which produces two protein isoforms, GRH.N and GRH.O, depending on the splicing of exon 4 and 5. In cell lineage tracing experiments, loss of both GRH.N and GRH.O results in a reduction in the number of progeny arising from a single ISC. Interestingly, loss of only the GRH.O isoform, which has previously been characterized as specific to neural tissue, results in an even greater reduction in progeny numbers. In the opposite experiment, we ectopically expressed both GRH.N and GRH.O isoforms in ISCs and its immature daughter cell, the enteroblast (EB). Ectopic expression of GRH.O resulted in an increase in ISC and EB numbers whereas GRH.N over expression resulted in the loss of the ISC and EB population. We suggest that GRH.O and GRH.N act in opposing manners to regulate ISC numbers. The mouse and human genomes contains three GRH orthologs, GRHL1-3. We have shown that GRHL2 and GRHL3 are both expressed in crypts within the mouse small intestine but GRHL3 is enriched in the stem cell pool and is upregulated during epithelial repair after challenge with 5-FU.

SYM-22-01

THE EVOLUTION OF DUAL ACTIVE SITES IN A DESIGNED ENZYME

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Developments in computational chemistry, bioinformatics and laboratory evolution have facilitated the de novo design and catalytic optimization of enzymes. Besides creating useful catalysts, the generation and iterative improvement of designed enzymes can provide valuable insight into the interplay between the many phenomena that have been suggested to contribute to catalysis. In this work, we have followed changes in conformational sampling, electrostatic preorganization and quantum tunneling along the evolutionary trajectory of a designed Kemp eliminase. We observe that destabilization of the active site leads to the emergence of a second, alternative active site geometry. Evolutionary conformational selection then gradually stabilizes the new active site, leading to an improved enzyme that is unlike the initial design. This work exemplifies the link between conformational plasticity and evolvability and demonstrates that residues remote from the active sites of enzymes play crucial roles in controlling and shaping the active site for efficient catalysis.
SYM-22-02

STABILITY ENGINEERING OF THE HUMAN ANTIBODY REPETOIRE

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Antibody therapeutics have transformed the treatment of cancer and many inflammatory conditions. Unfortunately, human antibodies often display limited stability and a propensity to aggregate, which has greatly hindered their development. We have identified hotspots of protein aggregation in antibody variable domains, and have developed generally applicable strategies to overcome stability issues. Here we outline the application of the technology to human antibody therapeutics, and present examples of how the approach can be utilized for the stabilisation of candidate molecules and libraries.

SYM-22-03

CYCLIC CELL PENETRATING PEPTIDES AS SCAFFOLDS TO TARGET INTRACELLULAR PROTEIN-PROTEIN INTERACTIONS

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Cyclotides, cyclic disulfide-rich peptides from plants, are ultra-stable molecules that have inspired applications in drug design as they can be used as scaffolds to stabilize linear bioactive sequences able to inhibit protein-protein interactions. Recently, they have also been shown to possess cell-penetrating properties. The combination of their remarkable stability and cell-penetrating properties opens new avenues for the application of cyclotides as a stable delivery system able to cross cell membranes and inhibit intracellular proteins involved in cancer pathways. To realize and optimize the application of cyclotides as a drug framework and delivery system, we studied their ability to enter mammalian cells. In particular, we have studied the internalization of two cyclotides: kalata B1, a cyclotide belonging to Mobius subfamily; and MCoTI-II, a cyclotide that belongs to the trypsin inhibitor family. We have designed and synthesized a series of kalata B1 and MCoTI-II analogues and conducted structure-activity relationship studies using surface plasmon resonance, nuclear magnetic resonance spectroscopy, mass spectrometry, confocal microscopy and flow cytometry. We have shown that kalata B1, a globally-neutral, membrane-active peptide, enters cells via both endocytosis and by direct membrane translocation. Both pathways are initiated by targeting phosphatidylethanolamine phospholipids at the cell surface. MCoTI-II is a positively-charged peptide and unable to bind to cell membranes that enters cells via endocytic pathways. Based on structure-activity relationship studies we have re-designed both MCoTI-II and kalata B1 to improve its internalization properties and ability to target cancer cells. Our mode-of-action studies and design efforts to improve cellular uptake show that cyclotides can be reengineered to stabilize a linear peptide and to optimize their internalization properties and highlight the potential of these peptides as drug leads for the modulation of traditionally ‘undruggable’ targets, such as intracellular protein-protein interactions involved in cancer pathways.

SYM-22-04

DISSECTING AND ENGINEERING PROTEINS THROUGH PHAGE-DISPLAYED EVOLUTION

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The Weiss laboratory develops new chemical tools to dissect biology at the level of atoms and bonds. Many projects in the lab apply phage display to access vast libraries of bacteriophage-presented peptides and proteins. From such libraries, binding partners to cancer-associated biomarkers have been selected. The resultant phage with the cancer-specific ligands have been wired into nanometer-scale electrical circuits for direct measurement of cancer marker levels in the early diagnosis of cancer. In other experiments, the lab developed new phage variants to extend phage display to include membrane-bound proteins. The approach allows the synthesis of membrane protein libraries for protein solubilization and dissection.

SYM-22-05

A MECHANISTIC INSIGHT INTO INSULIN A6-A11 DISULFIDE BOND IN REGULATING HORMONE ACTIVITY AND STRUCTURAL STABILITY

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We have explored the role of the insulin A6-A11 disulfide bond in its function and stability. The two key features of a disulfide bond are its flexibility that allows 20 possible configurations and its ability to undergo redox reaction. Here, we eliminate these features by substituting the A6-A11 cysteine framework with a non-interconvertible and non-reducible unsaturated carbon-carbon (dicarba) bond. Novel insulin analogues were synthesized through improved chemical synthesis and ring-closing metathesis methods, yielding a fully active cis isomer and an inactive trans isomer, suggesting redox is not required but A6-A11 bond orientation is important for receptor binding. In vivo testing of the cis isomer revealed rapid lowering of blood glucose in mice. A series of biophysical assays were performed to explain the isomers’ different biological activities and to relate these to their structural stabilities. A loss of in-solution structural stability of both isomers was measured by temperature and chemical (Gu HCl) denaturation studies. Using limited-proteolysis, mass spectrometry (MALDI) analysis and X-ray crystallography, we revealed the importance of the A6-A11 disulfide bond in regulating insulin stability and that insulin A chain flexibility is crucial for receptor engagement. In conclusion, our studies demonstrated the important mechanistic roles of the A6-A11 insulin disulfide bond in maintaining the optimal structure for function and stability. These findings open up new avenues for the future design of improved insulins.
SYM-23-01

NOVEL APPLICATIONS OF HIGH-THROUGHPUT CAPTURE SEQUENCING: HUNTING FOR VIRUSES THAT TRIGGER TYPE 1 DIABETES


Type 1 diabetes (T1D) is among the most common chronic diseases of childhood, affecting >140,000 Australians and more than half a million children (<14 years old) globally. It is well established that T1D results from a combination of genetic and environmental factors. Indeed, we and others have clearly demonstrated that enterovirus (EV) infections serve as prime environmental triggers of islet autoimmunity (IA) and T1D. However, the selective focus by most studies on EVs compared to all other viruses, using EV-specific detection methods (PCR or serology), suggest significant publication bias that may overestimate the strength of association between EVs and T1D. Therefore, our aim is to conclusively characterise the population of vertebrate viruses that promote the development of IA/T1D in at risk children, using comprehensive and unbiased virome capture sequencing (VirCapSeq-VERT). To date, we have completed VirCapSeq-VERT on 64 stool and 118 plasma samples from 45 case children (with IA) and age/sex matched controls from the Viruses In the Genetically at Risk (VIGR) Australian T1D birth cohort. Additionally, we have characterised the pregnancy gut virome of 61 mothers from the Environmental Determinants of Islet Autoimmunity (ENDA) prospective cohort, a nationwide study following 1,400 at risk infants from pregnancy to early life. Our preliminary analysis reveals significant differences in the virus population and viral read abundance between case and control children, as well as in mothers with T1D vs controls. This is the first study to utilise capture sequencing in investigating the virome during pregnancy, infancy and early childhood. Furthermore, it is the first to examine the virome in the Australian at-risk population, which may differ significantly from Europe/USA. We anticipate that with greater sample size and longitudinal follow-up, our data will inform and facilitate the development of vaccines that can prevent all viral triggers of IA/T1D.

SYM-23-02

BIG DATA FROM A SMALL DEVICE: REAL TIME GENOMICS WITH NANOPORE SEQUENCING

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Oxford Nanopore Technologies offer an affordable and portable sequencing platform that produces ultra long reads, and is capable of identifying epigenetic modifications in native molecules of DNA and RNA. On their own, these data can answer difficult questions in genome biology, but the technology offers more than ultra-long reads of native molecules. Here, we present some unique insights into genome biology from nanopore sequencing. We also elaborate on the real-time nature of nanopore sequencing, where biopolymer subsequences can be interrogated directly as they transit through the pore. To achieve this, we venture into ‘squiggle space’, a unique nanopore data format that precedes base calling. We describe how squiggles can be processed in real time to de-multiplex barcodes from single cell sequencing and perform transcriptome profiling on the fly. We envision that such real-time analyses will significantly improve turn around times in clinical settings.

SYM-23-03

THE OZ MAMMALS GENOMICS (OMG) INITIATIVE: DEVELOPING GENOMIC RESOURCES FOR AUSTRALIAN MAMMALS

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Australia has an incredibly diverse range of mammals, with species spanning all three major mammalian lineages. Unfortunately, Australia also has the highest mammal extinction rate in the world and many extant Australian mammals are listed as threatened. Consequently, we require a comprehensive understanding of the relationships of Australian mammals, including recently extinct species, to underpin studies of their evolution, as well as improve our understanding of extinction risk. It is now time that we capitalised on the advances in genome sequencing technology and bioinformatic analyses to enhance our understanding and conservation of Australia’s unique mammals. To facilitate the uptake of genomics for the conservation of Australia’s diverse and unique mammalian species, we have formed the Oz Mammals Genomics (OMG) consortium for the development of genomic resources for Australian mammals. The consortium consists of over 30 partners, including Australia’s natural history museums, university researchers and wildlife management agencies, with a one million dollar investment from BioPlatforms Australia, with co-investment from universities and museums nationwide. OMG aims to produce well-assembled marsupial genomes across the marsupial phylogeny using a combination of sequencing approaches, and to generate a comprehensive phylogenetic framework for Australian mammals (bats, rodents, marsupials) and population genomic datasets for threatened Australian species using predominantly exon capture. This presentation will discuss the different genomic technologies being used to achieve the aims of OMG.

SYM-23-04

TISSUE-SPECIFIC DELIVERY OF CRISPR-CAS ENDONUCLEASES FOR GENOME EDITING

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CRISPR-Cas RNA-guided endonucleases provide powerful capabilities to disrupt or correct genomic sequences through site-specific DNA cleavage and repair. However, both research and clinical uses of these enzymes are currently hampered by the lack of methods for cell- and tissue-selective delivery. We show that both Cas9 and Cpf1 ribonucleoprotein (RNP) complexes can be engineered for hepatocyte-selective gene editing upon receptor-mediated endocytosis. Receptor-based uptake requires both recognition of a ligand-enzyme conjugate and the presence of short pH-sensitive peptides that promote endosomal escape of RNPs for nuclear uptake. This strategy provides a framework for applications of gene editing in the liver and offers a general mechanism for targeted gene editing by receptor-mediated delivery of CRISPR-Cas complexes.
SYM-24-01
MOLECULAR REPORTERS FOR MEASURING UNFOLDED PROTEIN LOAD IN CELLS
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Maintaining proteostasis is an essential housekeeping function for cell survival. It involves chaperones and degradative pathways to ensure proteins fold correctly and to remove those that are misfolded, damaged or aggregated. In principle, proteostasis is affected in any disease that involves misfolded or mutant proteins that do not fold with normal efficiencies; and hence overdraw on the finite proteostasis resources of the cell. Tracking the proteostasis capacity of cells has the generic potential to track neurodegenerative diseases with diverse specific molecular origins. Thus building new approaches to identify the efficiency of proteostasis is highly desired in order to track the risk of cells succumbing to damage from protein misfolding and aggregation. In this project, a cysteine-reactive aggregation-induced emission (AIE) fluorescent probe was built for measuring the unfolded cell load and proteostasis capacity in cells. This probe, TPE-MI, can selectively detect solvent-exposed cysteines on intracellular proteins. Upon proteome folding stresses incurred by heat shock or tunicamycin, TPE-MI reactivity increased in accordance with an unfolded proteome. In cells expressing mutant aggregation-prone Huntington protein (associated with Huntington’s disease) there was a large increase in TPE-MI fluorescence prior to inclusion formation, suggesting that the collapse of proteostasis is one of the features for Huntington’s diseases and such an effect emerges in cells prior to the formation of visible aggregates. The formation of protein aggregates, which is a known common feature of neurodegenerative diseases, actually alleviates proteostasis stress. Our new approach to measure unfolded load provides a new capacity to probe proteostasis capacity in cells and mechanisms related to protein quality control which are of increasing importance in the research as well as for biomarker utility in early stage diagnosis of neurodegenerative diseases.

SYM-24-02
THERAPEUTIC TARGETING OF AUTOPHAGY IN ALS
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Autophagy is the main catabolic pathway in neurons that eliminates misfolded proteins, aggregates and damaged organelles associated with cellular stress, ageing and neurodegeneration. There is increasing evidence that autophagy is compromised in neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), which may contribute to cytoplasmic sequestration of aggregation-prone and toxic proteins in neurons. Genetic or pharmacologic modulation of autophagy to promote clearance of misfolded proteins may therefore be a promising therapeutic avenue. We screened clinically approved drugs and novel beclin-1 peptides for autophagy promoting activity using immunoblotting of macroautophagy (p62, LC3), chaperone-mediated autophagy (Hsc70, LAMP2A) and mitophagy (VDAC1) markers in neuronal cell lines and human pluripotent stem cell-derived motor neurons. Autophagic flux was determined using autophagy and mitophagy fluorescent reporter constructs. Autophagy inducer candidates were screened by treating transgenic RFP-EGFP-LC3 reporter mice. We identified a group of antidepressant, antipsychotic, anti-hypertensive and chemotherapy drugs which promote LC3II accumulation and autophagosome maturation shown by autophagic flux in vitro and in vivo. Of these autophagy enhancing agents, the anti-hypertensive drug rilmenidine was further evaluated in mutant superoxide dismutase 1 (SOD1) and TAR DNA binding protein 43 (TDP-43) mouse models of ALS for effects on clinical progression and motor neuron pathology. Rilmenidine treatment robustly upregulated LC3II and reduced TDP-43 levels in spinal cords of SOD1G93A mice, indicative of autophagy and mitophagy induction. Disease onset was not affected by rilmenidine, but survival was significantly reduced in SOD1G93A mice, correlating with accelerated motor neuron degeneration and increased aggregation of insoluble and misfolded SOD1 species and mitochondrial loss in motor neurons. These findings suggest that rilmenidine treatment may drive disease progression and neurodegeneration in this mouse model due to excessive mitophagy, warranting alternative agents and approaches to target autophagy in ALS.

SYM-24-03
PARKINSON-ASSOCIATED VPS35 MUTATIONS ALTER RETROMER CELLULAR FUNCTIONS
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Endosomal sorting is a highly orchestrated cellular process. Retromer is a heterotrimeric complex which associates with endosomal membranes and facilitates the retrograde sorting of multiple receptors, including the caten-independent mannose-6-phosphate receptor for lysosomal enzymes. The cycling of retromer on and off the endosomal membrane is regulated by a network of retromer-interacting proteins. Here, we find that Parkinson’s disease-associated Vps35 variant, R524W, and not P316S, is a loss-of-function mutation as marked by reduced WT SpCas9 mutated the second allele frequently (75%), a problem when targeting an essential gene. Our findings extend the toolbox of RNA-guided endonucleases for targeting essential genes.
SYMPOSIA  

WEDNESDAY

SYM-24-04

THE UNIQUE PROTEOSTASIS CHALLENGES OF PREGNANCY

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Pregnancy is a state in which physiological stresses that are capable of inducing protein misfolding are heightened. In part, this is a consequence of the systemic inflammatory response that is essential to gestation in mammals. Deposition of extracellular proteinaceous material known as fibrinoid occurs during healthy placenta development, but overproduction of fibrinoid is linked to intrauterine death, growth restriction, preterm delivery and gestational diabetes. Protein misfolding has also been implicated in the pathology of pre-eclampsia, a leading cause of pregnancy-related morbidity and mortality. Intuitively, mechanisms to counteract the accumulation of extracellular misfolded proteins will be enhanced in healthy women during pregnancy, but how this occurs is virtually unknown. In humans, alpha-2-macroglobulin (α2M) is a constitutively abundant secreted protein that is best known as a broad spectrum protease inhibitor. We demonstrated that the ability of α2M to stabilise misfolded proteins is dramatically increased by dissociation of the native α2M tetramer into dimers (Wyatt et al. 2014 PNAS, 20:111(20):E2081-90). This discovery prompted us to investigate pregnancy zone protein (PZP), which shares 71% sequence homology with α2M, but is a native dimer. Our unpublished results demonstrate that PZP efficiently inhibits amorphous and fibrillar protein aggregation. PZP is normally present at trace levels, but is markedly upregulated in pregnancy, and also in pregnancy-independent inflammatory states. We propose that PZP is an important element of the extracellular proteostasis network that is specifically upregulated to control protein misfolding in pregnancy and during chronic inflammation.

SYM-25-01

SUPPRESSING FATTY ACID UPTAKE AS A TREATMENT FOR PROSTATE CANCER

Taylor R.A.1, Clark A.K.1, Selth L.2, Rebello R.1, Porter L.A.1,2, Furic L.1, Risbridger G.P.1,2, Frydenberg M.1, Nomura D.K.1 and Watt M.J.3

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Altered metabolism is a hallmark of cancer pathogenesis and is required to support the malignant properties of cancer cells. Previous studies have focused extensively on the roles of glucose, glutamate and fatty acids derived from de novo lipogenesis in modulating the bioenergetic processes and macromolecule synthesis required to sustain growth and proliferation. Fatty acids are also derived from adipose tissue lipolysis or the breakdown of triglycerides contained in circulating chylomicrons and lipoproteins. Recent work from our laboratories shows that fatty acid uptake is increased in malignant human prostate tissue and that the influx of fatty acids leads to increased lipid storage. This process is regulated by molecular reprogramming of genes and proteins encoding lipid metabolism in human prostate cancer. Specifically, the expression of CD36, which encodes the major fatty acid transporter, is associated with reduced survival in prostate cancer patients. We have established CD36-mediated fatty acid uptake as a critical process for the production of lipid biomass and the generation of oncoprogenic signaling lipids in prostate cancer. Furthermore, we show that CD36 monoclonal antibody therapy reduces prostate cancer sensitivity in patient derived xenografts of high-risk localized disease, supporting the premise that blocking fatty acid uptake could be a promising therapeutic approach.

SYM-24-05

IN VIVO FUNCTION OF THE CHAPERONIN TRIC

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The TCP-1 Ring Complex (TRIC) is a multi-subunit group II chaperonin that assists nascent or misfolded proteins to attain their native conformation in an ATP-dependent manner. Functional in vitro studies have suggested that TRIC is an essential and generalized component of the protein folding machinery that folds up to 10% of the proteome. However, involvement of TRIC in specific cellular processes within multicellular organisms is largely unknown, as little validation of TRIC function exists in animals. Our in vivo analysis reveals a surprisingly specific role of TRIC in the biogenesis of skeletal muscle alpha-actin during sarcomere assembly in myofibers. Zebrafish possess single, highly conserved orthologs for each of the 8 different TRIC subunits that share 89.9% to 95.1% sequence identity with humans. Surprisingly, all homozygous cct zebrafish mutants develop into relatively normal larvae and all possess a highly specific defect in sarcomere assembly in skeletal muscle fibers. Mutations in cct specifically result in impaired folding of skeletal muscle alpha-actin at Z-disks, causing aggregate formation and reduced sarcomere assembly. These results suggest that TRIC acts as a protein scaffold enabling efficient skeletal muscle alpha-actin processing for thin filament assembly at the Z-disk. Additionally, characterization of a missense mutation in the ATP binding pocket of Cct6 suggests subunit specific regulation for the folding of skeletal muscle alpha-actin. Furthermore, TRIC regulates formation of protein aggregation and nemaline rod formation from mutated forms of skeletal muscle alpha-actin that result in human myopathies, implicating TRIC as a potential disease modifier in actin related myopathies.

SYM-25-02

AMINO ACID HOMEOSTASIS IN CANCER CELLS

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Amino acid homeostasis is crucial for cancer cells. Rapidly growing cells have high demands for building blocks, in particular amino acids. In addition to proteins synthesis, they are also building blocks for polynucleotide biosynthesis, lipogenesis, polyamine biosynthesis and other essential processes. The maintenance of amino acid pools in the cytosol is essential for the survival of cancer cells. Amino acid pools are controlled by amino acid transport, protein biosynthesis and degradation and amino acid biosynthesis and degradation. These processes are finely tuned by signalling networks such as mTORC1 and GCN2/ATF4. Deletion or silencing of amino acid transporters such as ASC12, SNA1 and SNA2 demonstrates a crucial role of these transporters in the support of amino acid pools and metabolic pathways. Our understanding of these processes has developed to the point where we can simulate amino acid fluxes to gain a better understanding of amino acid homeostasis.
SYM-25-03
THE USE OF STABLE ISOPE-RESOLVED METABOLOMICS TO ASSESS CANCER METABOLISM

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It is widely believed that to facilitate growth and uncontrolled proliferation, cancer cells undergo metabolic reprogramming which includes upregulation of de novo lipogenesis and protein synthesis. However, many of these observations are based on surrogate measures rather than the direct determination of metabolic fluxes. In this presentation, the application of stable isotopes, particularly deuterated water (H₂O), to determine the synthesis of DNA, protein and lipids in systems ranging from cells, animals and humans will be discussed.

SYM-25-04
ADAPTIVE REPROGRAMMING OF DE NOVO PYRIMIDINE SYNTHESIS IS A METABOLIC VULNERABILITY IN TRIPLE-NEGATIVE BREAST CANCER

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Treatment options for patients with triple-negative breast cancer (TNBC) are largely limited to conventional genotoxic chemotherapy agents. The long-term prognosis for TNBC patients with residual disease after chemotherapy is poor and a need exists to identify rational combination therapy approaches to improve the efficacy of chemotherapy for treating TNBC. Recent studies suggest that reprogramming of cellular metabolism is a component of the highly coordinated response to genotoxic stress. However, the metabolic response to clinically relevant genotoxic chemotherapy agents is poorly understood. We sought to identify adaptive metabolic reprogramming events triggered upon chemotherapy exposure that can be targeted to improve the efficacy of chemotherapy for treating TNBC. Using in vitro and in vivo metabolic profiling of TNBC cells, we have discovered an increase in the abundance of pyrimidine nucleotides in response to chemotherapy exposure. Mechanistically, the increase in pyrimidine nucleotides induced by chemotherapy is dependent on enhanced activity of the de novo pyrimidine synthesis pathway. We have found that pharmacological inhibition of de novo pyrimidine synthesis sensitizes TNBC cells to genotoxic chemotherapy agents by exacerbating DNA damage. Moreover, combining chemotherapy with leflunomide, a clinically approved inhibitor of the de novo pyrimidine synthesis pathway, induces regression of TNBC xenografts. Our studies provide pre-clinical evidence to demonstrate that adaptive reprogramming of de novo pyrimidine synthesis represents a metabolic vulnerability that can be exploited to improve the anti-cancer activity of genotoxic chemotherapy agents for the treatment of TNBC.

SYM-25-05
INHIBITION OF GLUCOSYLCERAMIDE SYNTHASE CAUSES MULTIPLE MYELOMA CELL DEATH ALONE AND IN SYNERGY WITH BORTEZOMIB VIA ENHANCED ENDOPLASMIC RETICULUM STRESS

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Ceramide is an apoptotic sphingolipid which is often elevated in cells by chemotherapy and radiotherapy, and contributes to the cell death caused by these agents. Some cancers are able to avoid the pro-apoptotic signalling produced by ceramide by upregulating enzymes which are able to convert ceramide to less apoptotic sphingolipids. Glucosylceramide synthase (GCS), which converts ceramide to glucosylceramide, is one such enzyme. One cancer in which GCS seems to be important is multiple myeloma, a currently incurable blood cancer which arises from plasma cells. GCS is significantly upregulated in patient myeloma cells compared to normal plasma cells. Inhibition of GCS, both genetically through shRNA, and pharmacologically through the GCS inhibitor PDMP, causes cell death of myeloma cell lines, as measured by flow cytometry with Annexin-V/PI staining. This cell death was accompanied by an increase in markers of endoplasmic reticulum (ER) stress and caspase-3 cleavage, suggesting the mechanism of cell death is apoptosis induced by enhanced ER stress. Furthermore, combining PDMP with the proteasome inhibitor bortezomb, which is current first line therapy for MM, is able to cause synergistic cell death of MM cell lines, even in cell lines that are bortezomb resistant, which was associated with synergistic induction of ER stress. Given that bortezomb resistance is a substantial hurdle in the treatment of MM, the ability of PDMP to improve bortezomb response in these cells is highly significant. Therefore, it seems that GCS inhibition may be a viable target in multiple myeloma.

SYM-26-01
ENGAGING STUDENTS WITH ANIMATION: AN ALTERNATIVE ASSESSMENT TASK

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Animation is increasingly being used by educators and biologists to demonstrate complex molecular processes that occur within cells that are difficult to visualise. There is also an increased use of animations on websites and presentations in many different disciplines. In 2016 we introduced a new unit in Molecular and Cell Biology where students were challenged to produce animations that explain key biological concepts for their major assessment task. Students worked in pairs to produce animations that accurately addressed scientific topics, were dynamic in visual presentation and contained narration. Learning was supported by a presentation from a renowned biomedical animator, animation skills workshops and a draft report where students received feedback. Final animations were presented in student groups where a diverse range of creative animations were presented. Student experience of the animation task was assessed by anonymous survey. Following analysis of this survey and staff feedback, some refinements of the task to support student learning and skill development have been implemented in 2017.
SYM-26-02
ENGAGING STUDENTS WITH ONLINE INTERACTIVE PRE-PRACTICAL CLASS ACTIVITIES
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It is well known that students who are prepared for class are more actively engaged in-class. A challenge to this for many university educators is student motivation and engagement with pre-class activities. An attempt to increase student engagement and promote active pre-class learning in an undergraduate molecular cell biology unit, a series of interactive online activities were developed using Articulate Storyline. These activities included a number of interactive elements and were designed to introduce students to background content as well as laboratory procedures and concepts. Student responses and engagement with pre-class activities were assessed using an anonymous survey that consisted of a mixture of Likert items and open-ended questions. Feedback from students (n=223) was overwhelmingly positive and showed that the students agreed or strongly agreed that the interactive format of the pre-class activities was engaging (77.5%) and that the activities prepared students for the practical class (81.5%). Student comments to open-ended questions further highlighted the positive impact of these activities and provided evidence of pre-class student engagement, which improved in-class engagement and learning.

SYM-26-04
AN INQUIRY BASED-LEARNING MODULE TO FOSTER CRITICAL THINKING IN A SECOND-YEAR BIOCHEMISTRY PRACTICAL CLASS
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The key attributes of science graduates include the ability to combine theoretical knowledge in a content area with practical application, critical thinking and trouble-shooting skills. All of these attributes are enhanced through the process of scientific investigation, which is the cornerstone of practical-based teaching. Unfortunately, there are distinct limitations to providing wet-lab inquiry-based learning opportunities in large classes within the early stages of a degree due to the logistical and financial impost intrinsic to such activities. To address this limitation, a student-centred inquiry-based learning module (set within the innovative Smart Sparrow platform) has been designed to integrate knowledge of theory with practical application, and to examine student understanding of experiment design and analysis. The learning module covers the common biochemistry and molecular biology techniques taught in BCMB20005: Techniques in Molecular Science (a second-year biochemistry practical subject) framed in such a way that there are three projects, each with a general theme to overexpress and purify a protein. Students must select a project to interrogate and examine, and are provided with lists of materials and equipment available to facilitate their decision making process. In this presentation, I will present an overview of the module with some examples of student activities designed to foster critical thinking and troubleshooting of experimental design.

SYM-26-03
IS TECHNOLOGY ENHANCING OR TRANSFORMING ASSESSMENT?
Macaulay J., Groessler A., Haynie A., Higgs B., Mercer-Mapstone L., Sweeney T., West D. and Yeo M.
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Assessment of learning is a critical component of education and is acknowledged to drive and guide student learning. Improved design and implementation of effective and innovative assessment strategies should therefore lead to improved student learning. Significant improvements are being implemented in higher education in relation to curriculum delivery but changes to assessment practice have been much slower. Hence, there is a need for assessment reform to mirror the current advances in both pedagogy and technology. This study examined how technology is being utilised for assessment, and to what extent, and how these technologies are enhancing or transforming assessment and feedback. The study was performed using a systematic literature review of peer reviewed journals. A survey instrument was designed to allow rigorous analysis of journal articles, including questions of: type of assessment, educational theory, pedagogical approach, educational goal, type of technology, technology use and affordances for assessment. The survey instrument design allowed for both quantitative analysis of the articles and qualitative assessment to capture the researchers insights. The results showed, that in the literature examined, technology is used extensively for formative and peer learning as part of the assessment cycle. The majority of articles examined reported using technology as a substitution for traditional assessment methods. However a significant minority of articles provided examples where technology is enabling assessment task redesign for transformation of learning.

SYM-26-05
ENHANCING FUNDAMENTAL BIOCHEMISTRY LABORATORY SKILLS THROUGH THE USE OF SIMULATIONS AND AN APP
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Biochemistry has a core foundation where key concepts are demonstrated through practical classes. For student learning, there are a hierarchy of skills that must be developed. These include comprehension of the principle(s) to be demonstrated, learning to use laboratory equipment and then linking theory with the experimental results. One key area where students experience difficulties is in learning to use new pieces of equipment, such as pipettes. Students struggle with choosing the correct pipette, setting the volume correctly and being able to deliver an accurate volume. Given the importance of pipettes in most laboratory settings, I developed an interactive simulation to teach students how to use a pipette. Students are then challenged to set a virtual pipette to a given volume, and provided with feedback on the choice of pipette and the setting used. A complementary mobile app (Pipette Master™) allows students to check the correct setting of a pipette for a given volume in the laboratory. The app covers pipettes ranging from 2μl to 5ml. The app allows the user to effectively operate all laboratory pipettes without damaging any equipment while learning. The app recreates a real-life situation, with the desirable outcome of no consequences for any mistakes. These digital approaches have begun to be evaluated with a 1st year Biology cohort. These students were taught to use automatic pipettes and then surveyed about their experiences. The same cohort will take Biochemistry in 2018, will be re-taught how to use the pipettes, but also given access to the simulation and the app during practical classes. The students will be re-surveyed to assess the impact of the new approaches. It is predicted that these digital technologies will enhance the learning of hands-on practical skills.

ComBio2017 • Adelaide, South Australia • 2-5 October, 2017
SYM-27-01
THE PATTERNING OF PLANT DEVELOPMENT - A STORY OF SELF-ORGANISING BOUNDARIES AND POLARITY

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Although plant organs such as leaves and flowers form a dizzying variety of shapes and configurations, certain features are common. For instance, leaves and floral organs are often flattened along their top-bottom axis. Also, such organs are typically positioned in a periodic manner, giving rise to spiral or whorled organ arrangements and fraxel leaf shapes. By using live-imaging and fine perturbation techniques, our research has started to reveal the developmental mechanisms underlying these common features. Our results demonstrate a central role for self-organisation both at the level of cell-cell interactions as well as between tissue types. Further details will be presented.

SYM-27-02
RICE WITH MULTILAYER ALEURONE AND ENHANCED MICRONUTRIENTS

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The rice aleurone is the outer specialized layer of the endosperm, normally a single cell layer, which is particularly rich in nutrients. A forward genetic screen of a rice mutant population by colleagues in the Institute of Botany, Chinese Academy of Sciences, found a number of mutants with multilayer thick aleurone. The gene responsible for one of the mutations has now been determined through fine mapping. The gene is REPRESSOR OF SILENCING 1, ROS1, and the mutation occurs in an intron causing alternative splicing to translate a protein with an extra seven amino acids. ROS1 appears to be a bi-functional enzyme with DNA gyrase activity removing 5-methylcytosine, and lyase activity nicking double-stranded DNA at abasic sites. Previously a maternal knock-out allele in rice was shown to cause failure of endosperm development, even in the presence of the wild type paternal allele. Six other thick aleurone mutations in ROSTA have now been isolated by TILLING at IB CAS, none of which are knock-outs. The TILLING was targeted to a different region to the original mutation. Wholemeal flour prepared from field grown plants had substantial increases in lipid, B vitamins, antioxidants, minerals and fibre. The increased sink size of aleurone has enabled greater accumulation of components normally found in the aleurone. Cells in the thick aleurone maintained typical aleurone characteristics, thick cell wall, abundant protein “grains” and lipid bodies. There was 66% increase in total dietary fibre, increases in phenolic compounds and antioxidant capacity, and aleurone-grain associated minerals, niacin and phytate. The three ROSTA genes of allohexaploid wheat have been isolated and sequenced. Sixteen ROS1A gene mutants including missense and stop-gain mutations, have been isolated from TILLING populations. These mutations are being studied and combined in an attempt to reproduce the thick aleuron phenotype in wheat.

SYM-27-03
ROLE OF LEUNIG AND LEUNIG_HOMOLOG IN REGULATING EARLY EMBRYONIC PATTERNING IN ARABIDOPSIS

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Establishment of the apical-basal axis during the early stages of plant embryogenesis is necessary for root and shoot formation. While transcriptional regulators that specify cell fate along this axis are known, our understanding of this process remains incomplete. Recently we found that the transcriptional corepressors LEUNIG (LUG) and LEUNIG-HOMOLOG (LUH) play an important role in embryonic shoot formation. This is based on the observation that lug luh double mutant embryos display aberrant cell divisions in the apical region during early embryogenesis and fail to form a shoot. This phenotype is correlated with an altered distribution of auxin, a hormone involved in apical-basal patterning, as well as altered expression of transcription factors that specify cell fate. Although functioning as transcriptional regulators, LUG and LUH cannot bind DNA directly, instead relying on physical interactions with the co-regulators SEUSS (SEU) and SEU-LIKE (SLK) proteins, which themselves associate with a variety of transcription factors. Using a combination of yeast and plant assays we show that both SEU/SLK and LUG/LUH proteins physically interact with WUSCHEL-RELATED HOMEOBOX (WOX) transcription factors. These interactions are highly significant, as WOX transcription factors promote both axis formation and cell fate specification during embryogenesis. We report on experiments analyzing genetic interactions between the LUG/LUH co-repressors and WOX transcription factors. We also present work showing how the LUG-LUH regulatory complex promotes shoot formation via the class III HD-ZIP transcription factors. Based on these observations we present a model for how the basic body plan of the plant is established.

SYM-27-04
LOFSEP MADS-BOX GENES DETERMINE PANICLE ARCHITECTURE AND SPIKELET IDENTITY IN RICE

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SEPALLATA (SEP)-like genes, which encode a subfamily of MADS-box transcription factors, are essential for specifying floral organ and meristem identity in eudicots. The monocot model species rice (Oryza sativa.), has five SEP-like genes with partial redundancy and overlapping expression domain. Here, we describe the biological role of three SEP genes of rice, OsMADS1, OsMADS5 and OsMADS34, which represent the conserved LOFSEP subgroup, in redundantly controlling spikelet morphogenesis. Strong expression of the three genes was detected across a broad range of reproductive stages and tissues. No obvious phenotype was observed in the osmads5 single mutant when compared with the wild type, which was largely due to the functional redundancy among the three LOFSEP genes. Genetic and molecular analysis demonstrated that OsMADS1, OsMADS5 and OsMADS34 together regulate floral meristem determinacy, and specify the identities of spikelet organs by positively regulating the other MADS-box floral homeotic genes. Experiments conducted in yeast also suggested that OsMADS1, OsMADS5 and OsMADS34 form protein-protein interactions with other MADS-box floral homeotic members, which seems to be a typical, conserved feature of plant SEP proteins. In addition, OsMADS34 is also a regulator of inflorescence architecture, which is an important determinant of yield. The possible involvement of OsMADS1 and OsMADS5 in this process, and the downstream regulatory pathway are currently under investigation, in rice and also in barley.
Gene editing technologies rely on endonucleases to generate double stranded breaks (DSBs) at target loci. The DSBs are repaired through the error-prone non-homologous end joining (NHEJ) and homology-directed repair (HDR) pathways in cells, resulting in mutations and sequence replacement, respectively. In the widely used CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats/CRISPR-associated Cas9) system, the endonuclease Cas9 is targeted by a CRISPR small RNA to DNA sequence of interest. I will highlight the use of CRISPR/Cas9 and CRISPR/Cpf1 for generating mutant alleles for plant functional genomics research. I will also describe recent developments based on CRISPR/Cas9 for precise base editing and gene targeting that can be powerful tools for precision molecular breeding in crops.
CRISPR loss of function screens provide a highly specific approach to modulate gene expression and permit to systematically study how genes function in normal conditions and how they are deregulated during disease. However, cost, labor, and the requirement for specialized equipment limit the use of arrayed screening approaches. Recent technological advances and reduction in sequencing costs are enabling cost efficient high-quality pooled genome wide CRISPR screens and are revolutionizing our understanding of gene function and identification of drug targets. Although we have identified few CRISPR mediated off target effects we demonstrate the utility of combining WT or catalytically inactive Cas9 (dCas9) we to mitigate these effects. Furthermore, to gain deep unbiased mechanistic insights into gene function we have recently developed an approach for medium throughput mechanistic studies that includes genetic interaction mapping and large-scale RNA-Seq following CRISPR mediated suppression of hundreds of genes. Although my work uses these approaches towards understanding and targeting of cancer these approaches could be used as a general strategy to study gene function.

Pentatricopeptide repeat (PPR) proteins are modular single-stranded RNA binding proteins with potential as tools to modulate gene expression at the RNA level. Our earlier work defining the amino acid “code” for RNA specificity identified residues 5 and 35 of each 35 amino acid sequence repeat as directing base specificity. Recent studies of “designer PPRs” by a number groups and associated structural data have shed some light on the detail of the mode of binding of this class of protein to RNA. There has been less success however in translating this knowledge into a designer PPR that is programmed for a non-redundant RNA sequence of biological relevance. Here we report a designer PPR that binds in vitro with specificity to a known native PPR RNA target. Our 2.4 Å structure shows a spring-like contraction of the PPR super-helix upon RNA binding, in contrast with our previous reported 2.2 Å model of the unbound protein. Gel filtration studies confirm this substantial contraction of the PPR super-helix in solution. The striking conformational change is explained by steric constraints placed on the protein-RNA complex in which binding of extended tracts of RNA bases is only possible in the contracted state. This has implications for future designer PPR projects and provides insight into the mechanisms and dynamics of PPR-RNA binding.
SYM-29-04

TRACKING Oligomeric transcription factor dynamics by pair correlation of molecular brightness (pCOMB)

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Oligomerisation of transcription factors controls their translocation into the nucleus and DNA binding activity. Here we present a fluorescence microscopy method termed pCOMB (pair correlation of molecular brightness) that tracks the mobility of different oligomeric species within live cell nuclear architecture (Hinde et al. 2016. Nature Comm. 7(11047)). pCOMB amplifies the signal from the brightest species present and filters the dynamics of the extracted oligomeric population being measured, arriving at differences between two locations. Here we use this method to demonstrate a dependence of signal transducer and activator of transcription 3 (STAT3) mobility on oligomeric state. We find that upon entering the nucleus STAT3 dimers must first bind DNA to form STAT3 tetramers, which are also DNA-bound but exhibit a different mobility state. Examining the dimer-to-tetramer transition by a cm can pair correlation analysis (cpCOMB) reveals chromatin accessibility to modulate STAT3 tetramer formation. Thus the pCOMB approach is suitable for mapping the impact oligomerisation has on transcription factor dynamics.

SYM-29-05

CRYSTAL STRUCTURES OF AN UNUSUAL TRANSCRIPTIONAL ACTIVATOR FROM BACTERIOPHAGE 186

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The temperate bacteriophage 186, after infecting its host bacterium Escherichia coli, can follow either the lytic or the lysogenic developmental pathways. Crucial to this developmental decision is the lysozyme promoting factor CII. This potent transcriptional activator activates the early lysozymic promoter pL at least 400-fold, to build up sufficient immunity repressor levels for a portion of infections to commit to lysogeny. Its potency and its unusual property of binding to half sites separated by 20 base pairs, center-to-center, suggests it may activate the pL promoter by a novel mechanism. Three crystal structures of the CII protein were solved to 2-3Å. The structures reveal that a tetrameric arrangement of CII is necessary for DNA binding, which was subsequently validated by mutational analysis and native mass spectrometry. CII is degraded in vivo into a specific transcriptionally inactive product. The crystal structures explain the altered self-association of the degradation product and its loss of activity. The structures combined with mutagenesis data provide a basis for modeling the CII-RNA polymerase complex at the promoter to aid in understanding the promoter activation mechanism.

SYM-30-01

TRANSLATING CANCER GENOMICS TO THE CLINIC, FINDING ADVANCED CHILDHOOD AND RARE ADULT CANCERS


The comprehensive identification of genetic alterations in patient tumours offers the opportunity to substantially improve patient care, through identifying optimal therapies, obtaining a more precise diagnosis, and identifying inherited cancer risk variants. Patients with rare (collectively 30% of cancer deaths), or advanced cancers stand to benefit substantially from a genome-guided approach, whereby rational predictions, or proven genotype-drug combinations from well-studied cancers can be transferred to patients with otherwise under-served. We have established the Molecular Screening and Therapeutics (MoST) program. The unique design combines a molecular screening platform to identify actionable variants under an overarching protocol for multiple, parallel, signal-seeking clinical substrates. Patients are screened using a 387-gene targeted sequencing panel, and enrolled onto treatment studies matched to tumour genotype (eg CDK4/6 amplification and Palbociclib). In 11 months, we have profiled 225 patients on the panel Australia wide, 28% of patients being enrolled onto a substudy (including immunotherapy). Furthermore, we identified actionable variants in 19% of patients on a MoST substudy, and 14% of with a treatment recommendation outside of MoST (eg HER2, or ROS inhibitors), supporting the wide utility of genomic screening in rare cancers. In addition, we have established the Lions Kids Cancer Genome Project (LKCGP), wherein we are using deep whole genome sequencing to deliver individual treatment recommendations to patients enrolled on the Zero Childhood Cancer (ZCC) national child cancer personalised medicine program, being led by Children’s Cancer Institute and Sydney Children’s Hospital. In the past 12 months, of the first 45 patients recruited, we have identified at least one clinically relevant finding to the molecular tumor board in 73% of cases. These findings include treatment recommendations, identification of germline risk variants in 5 cases, and change of diagnosis and thus patient management in two patients. In this presentation, we will present our results and methodology for translating genomics to the clinic, in real time, for patients with advanced childhood and rare adult cancers.

SYM-30-02

IDENTIFYING HETEROGENEITY IN CANCER SAMPLES USING WHOLE GENOME SEQUENCING

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Personalized medicine, whereby a patient’s tumor is genetically tested and then specifically treated with a tailored therapy, has the potential to revolutionize cancer treatment to the benefit of patients and the Australian economy. It is well known that temporal and spatial intra-tumor heterogeneity occurs in most cancers so the practice of characterizing a small part of the primary tumor to infer therapy does not capture the genomic differences that can exist in other regions of the same tumor, and in particular metastatic deposits. These genomic differences mean that some cells in the tumor can be resistant to the targeted therapy and can limit the clinical realization of personalized medicine for cancer patients. Therefore systems for the detection and characterization of the different sub-clonal genomic variants in both primary and advanced disease are required to improve patient outcomes. In this talk I will discuss our efforts using whole genome sequencing to detect and characterize heterogeneity within temporally and spatially separated tumor samples across a range of tumor types.
INTEGRATIVE GENOMICS AT DIAGNOSIS OF CHRONIC MYELOID LEUKAEMIA REVEALS MOLECULAR HETEROGENEITY THAT MAY UNDERLIE DIVERSE TREATMENT OUTCOMES

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Chronic myeloid leukaemia (CML) represents the prototype of genetically based diagnosis and management, and tyrosine kinase inhibitors (TKIs) exemplify the success of molecularly targeted therapy. TKIs target the primary genetic lesion common to all patients, which is the BCR-ABL1 fusion oncoprotein. Prior to the TKI era, transformation to a rapidly fatal acute leukaemia invariably occurred at a median of 3 years after diagnosis. Most patients now have long-term survival, however 10-20% fail therapy. The most common mechanism of acquired TKI resistance is mutation within the BCR-ABL1 kinase domain that interferes with drug binding, but these are rarely responsible for primary drug resistance. The genomic evidence indicating BCR-ABL1-independent resistance and early disease transformation remain poorly understood. Identification of patients at diagnosis who are destined for poor outcome with standard therapy may guide investigational studies of novel therapies. We hypothesized that genetic lesions in addition to BCR-ABL1 are present at diagnosis that modify response. Whole exome/transcriptome sequencing coupled with copy number variation analysis was performed to uncover the somatic mutations that may underlie disease transformation. The diagnosis samples of patients diagnosed in the relatively chronic phase who had a rapid response to TKIs were compared to chronic phase patients with subsequent rapid disease transformation at a median of 6 months after diagnosis. There was a significantly higher frequency of clinically relevant mutations at diagnosis in the patients with rapid transformation (66% vs 17%). Furthermore, integrative genomics identified additional clinically relevant mutations in all patients at the time of transformation. Novel, recurrent copy number variations and mutated genes were identified. Histone methyltransferases were among the most frequently mutated and represent promising future drug targets since small molecule inhibitors are in development. Future refined biomarker testing of specific variants may provide prognostic information and inform therapy decisions and drug development.

MICRORNA-200/375 REGULATED QUAKING AND DRUG DEVELOPMENT.

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Epithelial mesenchymal transition (EMT) contributes to metastatic cancer progression and is regulated by complex transcriptional and post-transcriptional changes. Members of the miR-200 family are critical gatekeepers of the epithelial state, restraining expression of hundreds of pro-mesenchymal genes. We report here that downregulation of miR-200c and miR-375 during EMT provokes widespread changes in alternative splicing (AS) through translational de-repression of a single RNA binding protein, Quaking 5 (QKI-5). QKI-5 directly regulates hundreds of EMT AS events converging mainly on targets within the actin cytoskeleton network, and alters cell plasticity without appreciably affecting mRNA levels. QKI-5 driven AS exerts pleiotropic effects, such as stimulating cell migration and invasion while restraining tumour growth. Notably, several actin-associated genes, including MRIP, NIN and MYO1F, are directly targeted by both miR-200c and QKI-5, revealing coordinated control of mRNA abundance and AS during EMT. Furthermore, QKI-5 driven, EMT-associated AS signatures are broadly evident across many cancer types indicative of conserved functions and, consistent with these results, QKI-5 is associated with poorer prognosis in cancer patients. These findings demonstrate the existence of a miR-200/375-Quaking axis that globally controls alternative splicing and critically impacts on cancer-associated epithelial cell plasticity and cancer malignancy.

FROM MAMMALIAN DEVELOPMENT TO CANCER: THE ENCOMPASSING ROLE OF A TUMOUR SUPPRESSIVE PHOSPHATASE

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Protein Phosphatase 2A (PP2A) is a serine/threonine phosphatase, fundamental for cell proliferation, survival and differentiation, via its regulation of cell division kinases/signalling pathways such as RAS/ERK and PI3K/AKT. Inactivation of PP2A is a common event in human cancers. PP2A is comprised of a structural A-subunit, catalytic C-subunit and variable regulatory B-subunit, which directs substrate specificity and subcellular targeting. Recent genome sequencing efforts have identified that deletion of the PP2A-B55α regulatory subunit (encoded by the Ppp2r2a gene) occurs in ~17% of breast cancers and is associated with poor prognosis. However, little is known regarding the functional role of Ppp2r2a in normal mammalian physiology or breast tumourigenesis. Therefore, here we generated the first Ppp2r2a knockout mouse model using CRISPR/Cas9 technology, to address this gap. Across two independent CRISPR lines on a C57Bl6 genetic background, of 70 litters from heterozygous breeding pairs, we have had 274 pups, with 36% wildtype (Ppp2r2a+/−) and 64% heterozygotes (Ppp2r2a−/−), but no pups with homozygous deletion (Ppp2r2a−/−). Thus constitutive Ppp2r2a knockout is embryonic lethal. The block in development occurs after embryonic day 18.5, much later than catalytic or structural subunit knockouts (E6.5 and E10.5, respectively), suggesting a specific role for B55α containing PP2A. Heterozygous null and late-developed adult heterozygous mice showed decreased PP2A-B55α protein expression in all organs analysed, especially mammary glands. Ppp2r2a−/− mice also displayed reduced expression of PP2A inhibitory protein CIP2A and decreased ERK activation, compared to wildtype. Structural examination of mammary glands further revealed significantly decreased branching in Ppp2r2a−/− mice, suggesting a key role for PP2A-B55α in mammary gland morphogenesis. These results highlight the vital role of PP2A-B55α in normal mammalian embryonic development, and now provide a powerful model to elucidate the functional role of PP2A-B55α loss in breast cancer.

MICRORNA-200/375 REGULATED QUAKING CONTROLS EPITHELIAL CELL PLASTICITY THROUGH WIDESPREAD ALTERNATIVE RNA SPLICING

SYM-30-05

SYM-30-04

SYM-30-01

SYM-30-30

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SYM-31-02

NPPA AND NPPB FUNCTION REDUNDANTLY IN THE ZEBRAFISH EMBRYO TO RESTRICT THE AVC AND SYNTHESIS OF CARDIAC JELLY

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Natriuretic peptide type A (nppa) and type B (nppb) are clinical markers of hypertrophy and heart failure. In the embryo, nppa expression is used to label differentiating chamber myocardium and is notably restricted from the developing atrioventricular canal (AVC) and sinnataloid area. In mouse, Nppa or Nppb loss-of-function is associated with adult onset phenotypes (hypertension and cardiac fibrosis, respectively) however the functional role of these peptides during embryonic development is unclear. The two ancestrally related genes reside as a gene cluster adjacent to one another in the genome, making the creation of double mutants by homologous recombination a low probability. Using genome-editing approaches, we generated nppa and nppb single and double mutants in the zebrafish model. No phenotype was observed in single nppa- or nppb- mutants however by 3 days post fertilization (3 dpf), double mutants exhibit cardiac oedema, indicating poor cardiac function. Analysis of high-speed movies showed a thickening of the space between the inner endocardial and outer myocardial layers of the heart, suggesting increased cardiac jelly as the cause of impaired function. In situ analysis of AVC markers showed expansion of bmp4, tbx2b and has2expression into the chambers in double mutants compared with siblings. has2 is a synthetic enzyme of Hyaluronic acid (HA), a major constituent of the cardiac jelly, which may explain the increase in jelly thickness. We confirmed the increase in the cardiac jelly by crossing the double mutant carriers to a novel HA biosensor and observe significantly higher fluorescence intensity of the cardiac jelly of double mutants compared with siblings. Together, these data suggest that nppa and nppb function redundantly to restrict the AVC and cardiac jelly synthesis in the cardiac chambers, revealing a role for this gene cluster in cardiac development.

SYM-31-03

IMPROVING DIAGNOSTIC AND THERAPEUTIC IMAGING IN CHRONIC INFLAMMATORY DISEASES

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Clinical detection of chronic inflammatory diseases such as cancer and atherosclerotic plaques, relies on in vivo imaging technologies including positron emission tomography (PET) and magnetic resonance imaging (MRI). These technologies are particularly useful to track down and image the anatomy of pathological tissues, and provide some information on the disease heterogeneity. However, they are still technically limited with respect to spatial and temporal resolution and contrast generation. The uptake of radiolabelled tracer for PET, 18F-fluorodeoxyglucose (FDG), by tumour cells and inflammatory cells in plaques, is not only inconsistent but it also accumulates in normal cells that are metabolically active. Similarly, the use of iron nanoparticles for MRI contrast is ineffective strategy largely because the particles are not taken up efficiently in the pathological tissues but sequestered non-specifically in normal tissues. To provide better indication on the developmental stages and biological characteristics of the pathological tissues, contrasts agents can be tagged with tracers that specifically target the abnormal components of the disease, including vascularity, fibrosis and matrix formation, metabolic activity and inflammatory status. We have recently developed several molecular-targeted imaging contrast agents and novel procedures to enhance the detection of pathological lesions: 1. In vivo PET imaging of atherosclerotic plaques can be significantly enhanced using radiotracers coupled to a peptide that specifically binds inflammatory macrophages localised within rupture-prone plaques. 2. MRI detection of tumours can be greatly improved using an iron-nanoparticle payload coupled to a peptide that specifically targets the extracellular matrix in cancers. 3. In desmoplastic cancers that are impenetrable to molecular contrast agents, a therapeutic intervention to break the physical barrier is required. We have developed a new targeted drug and technology to soften the tumours for effective delivery of imaging agents and therapeutics.

SYM-31-04

FAST MOVING NEPHRON PROGENITORS ESCAPE COMMITMENT AND RE-ENTER THE NICHE

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Mammalian nephron progenitors are located within the cap mesenchyme that surrounds each tip of the ureteric epithelium to form a self-renewing progenitor niche. Throughout kidney development some progenitors differentiate in response to inductive cues and exit the niche to form early nephrons. The cap mesenchyme population is highly dynamic with cells migrating both within and between domains in response to cues from the niche. The process by which this motile population proceeds through mesenchymal to epithelial transition to form early nephrons in a precise, spatially regulated manner is not well understood. We activated a fluorescent reporter using Wnt4-CreERT2, a tamoxifen-inducible marker of terminal commitment, and observed labelling within differentiating progenitors as well as a proportion of apparently undifferentiated cap cells. Whole organ imaging analysis revealed a Wnt4 lineage that originates at sites of differentiation, but re-enters the cap over time. We observed cells undergoing niche re-entry in live time lapse imaging, and an increase in migration speed in the Wnt4-Cre labelled population compared to cells labelled with progenitor marker Sox-Cre. Thus fast moving nephron progenitors appear to escape commitment by limiting their exposure to inductive signals. We propose a model whereby moving cells commit based on time spent within an inductive region, leading to ongoing niche exit and re-entry events. Experimental results confirm an accumulation of labelled cap cells over time, as predicted by our model, highlighting a possible source of feedback from nephrogenic events to the niche.

SYM-31-05

STRUCTURAL CHARACTERISATION OF P-REX1 ACTIVITY IN CANCER CELL SIGNALLING

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P-REx1 is a guanine nucleotide exchange factor that activates a number of Rho family GTPases that are integral in regulating cell growth and motility. Recently, P-REx1 has been implicated in a number of cancer signalling pathways and as an important signal integrator in metastasis and abnormal cell growth. However, our current mechanistic understanding of the activation and regulation of P-REx1 is limited. Crystallisation and structural analysis of the catalytic DH-PH domains of P-REx1 have allowed the elucidation of its mechanism of nucleotide exchange. This mechanism was probed in a cell-based assay, where a FRET-biosensor was used to measure precise temporal changes in P-REx1 activity in live breast cancer cells. Mutational analysis of the P-REx1 active site in this assay confirmed its importance in multiple signalling pathways, highlighting its potential as a therapeutic target. Further, the regulatory role of P-REx1 C-terminal domains has been probed through cross-linking coupled with mass spectrometry. Through comparison of the free, inactive state of P-REx1 and its effector-bound, active state, we have discovered key intramolecular interactions that distinguish the two and provide insight into how P-REx1 is regulated in cells.
SYM-32-01

FUNCTIONAL AND MOLECULAR CHARACTERISATION OF INSULIN SECRETORY GRANULES
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Type 2 diabetes (T2D) is characterised by a loss of pancreatic beta-cell function. This presents mainly as a reduction in glucose-stimulated insulin secretion (GSIS). In beta-cells, mature insulin is packaged and stored in secretory granules (SGs). Upon stimulation, these granules mobilize and fuse with the plasma membrane, delivering insulin to the bloodstream. The basic machinery responsible for this regulated secretion consists of specific membrane proteins on SGs and the plasma membrane. Thus, the molecular composition of SGs can control their secretion properties. It is now accepted that SGs exist in two functionally distinct pools; newly synthesized SGs that are preferentially secreted upon stimulation, and older less mobile SGs that are preferentially targeted for degradation. How a cell can distinguish young SGs from old is unclear. Interestingly, under conditions of metabolic stress, beta-cells lose their ability to distinguish young SGs from old, hypersecretes insulin to compensate for the insulin resistance and become de-granulated. Whether these changes are at the level of the granule or at the level of the beta-cell environment is not determined. We have developed a method to physically separate age distinct insulin SGs and are using unbiased approaches to determine the molecular basis for the functional differences between these two SG pools, under normal and stress conditions in mouse islets. These studies will enhance our basic understanding of the distal steps of the insulin secretory pathway and provide strategies for restoring functionality of SGs as a potential therapeutic target for T2D.

SYM-32-02

IDENTIFICATION OF NOVEL PROTEIN REGULATORS OF LIPID METABOLISM USING A MULTI-OMICs RESOURCE
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Hepatosteatosis underlies several diseases including type 2 diabetes, cardiovascular disease and liver disease. Unfortunately, our understanding of the contributing pathways that initiate and advance hepatosteatosis to subsequent complications is poorly understood. Here, we take advantage of recent developments in omics technologies to perform high resolution proteomics (>5000 proteins) and quantitative lipidomics (>300 lipids) on livers from 107 genetically diverse inbred mouse strains from the UCLA hybrid mouse diversity panel (HMDP). Subsequent analyses demonstrate a striking 27-fold difference in hepatic triglyceride content across the strains in male mice fed a normal chow diet, which was associated with marked, yet specific alterations in proteome and lipidome signatures. Once integrated with genomic and phenomic datasets we were able to identify several pathways and proteins associated with a genetic propensity to store triglyceride. These included the well-known lipid droplet protein perilipin 2 (plin2) and a novel dehydrogenase called acyl-CoA dehydrogenase family member 11 (acad11). Of particular interest was the identification of a protein that was specifically associated with the abundance of pathological short-chain saturated diacylglycerol species. Subsequent validation in mice demonstrates that the human and mouse variant of this protein modulates the abundance of DGs in two separate strains of mice, suggesting a conserved and potentially therapeutically relevant role in regulating these pathological lipids.

SYM-32-03

INTEGRATIVE OMICS ELUCIDATES BIOLOGICAL PROCESSES UNDERLYING THE DISEASE AND MORTALITY RISKS OF THE BIOMARKER GLYCA
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Integration of electronic health records with systems-level biomolecular information has led to the discovery of robust blood-based biomarkers that predict future health and disease. The GlycA biomarker predicts long-term risk of diverse outcomes, including cardiovascular diseases, type 2 diabetes, and all-cause mortality. It is an agglomeration of five circulating glycoprotein concentrations: alpha-1-acid glycoprotein (AGP), alpha-1 antitrypsin (AAT), haptoglobin (HP), transferrin (TF), and alpha-1-antichymotrypsin (AACT), each of which dynamically responds over different times scales, directions, and magnitudes as part of the inflammatory response. In two recent studies we characterised biological processes associated with elevated GlycA levels in over 12,400 adults across two independent population-based cohorts. We found elevation of GlycA persisted for up to a decade within individuals, correlated with elevation of 29 inflammatory cytokines and correlated with a reproducible gene coexpression network indicative of increased neutrophil activity. Accordingly, analysis of infection-related hospitalization and death records in 7,599 adults showed that increased GlycA increased 14-year risk of severe non-localized and respiratory infections, particularly septicemia and pneumonia. Using a machine learning approach, we developed accurate imputation models for predicting the concentrations of AGP, AAT, and HP from serum NMR data and cohort metadata. Estimation of glycoprotein levels in 12,418 adults across two independent population-based cohorts revealed AAT had the strongest and broadest effects on 8-year disease incidence and mortality risk. Transcriptional analyses revealed elevated AAT corresponded to elevation of both innate and adaptive immune response pathways. In total, our work shows that elevated GlycA levels likely reflect a state of sub-clinical chronic inflammation and elevated immune response.

SYM-32-04

NUTRITION REGulates TRAFFICKING OF AMYLOID PRECURSOR PROTEIN TO THE LYSOSOME
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Amyloid-β (Aβ) accumulates in the brain during Alzheimer’s disease and impairs neuronal function. Aβ is produced by the sequential cleavage of amyloid precursor protein (APP) by secretases. However, APP itself can be trafficked to the late endosome/lysosome where it is destroyed by powerful lysosomal hydrolases. We hypothesised that lysosomal trafficking of APP could provide molecular targets for the treatment of Alzheimer’s disease. We therefore aimed to create a tool that could be used to quantitatively measure the trafficking of APP to the lysosome. APP695 was fused to mCherry and green fluorescent protein (GFP) to create a tandem fluorescent:APP (tfAPP). Flow cytometry was used to quantify the ratio of red fluorescence/green fluorescence (R/G) in a monoclonal HeLa tfAPP expressing cell line. When trafficked to an acidic environment (late endosome/lysosome), GFP quenches while mCherry does not, resulting in increased R/G. Flow cytometric measurement of R/G revealed that starvation induces autophagic flux (measured by tf-LC3) and also traffics APP to the lysosome. Inhibiting mTOR activity using AZD8055 had the same effect as starvation, inducing lysosomal trafficking of APP. Interestingly, whereas activation of mTOR by RHEB over-expression dramatically inhibited autophagic flux, RHEB expression did not affect lysosomal trafficking of APP. We demonstrate trafficking of APP to the lysosome can be measured using tfAPP. Whereas APP usually cycles between the plasma membrane, the early endosome, retromer and the Golgi apparatus, we show the effect of starvation through mTOR signalling can divert APP to the lysosome for hydrolisis by lysosomal proteases.
SYM-32-05
TARGET OF RAPAMYCIN (TOR) INTEGRATES ENVIRONMENTAL SIGNALS TO CONTROL CELL GROWTH AND DIVISION

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In order to continue to meet the demands of proliferation under environmental stress, cells have to coordinate their growth and division with available resources. The target of rapamycin (TOR) acts as a major nutrient sensor and is involved in regulating the coupling of cell growth and cell cycle progression in response to alterations in the nutrient environment. Comparative biology has been particularly revealing in understanding signalling networks. The fission yeast S. pombe and budding yeast S. cerevisiae diverged approximately 350 million years ago. S. pombe is a particularly excellent model for the study of cell growth and cell division as, unlike S. cerevisiae, S. pombe has retained through evolution complex heterochromatin, the RNAi machinery, large centromeres, gene splicing, telomere function and conserved checkpoints among others. While the TOR kinases were first discovered in S. cerevisiae, its upstream regulators TSC1/TSC2 that are regulated by AMPK signalling in most eukaryotic cells are conserved in S. pombe but not found in S. cerevisiae. Together, this makes S. pombe an ideal model system with which to establish core conserved principles of environmental control of TOR signalling and cell proliferation. We exploit S. pombe to uncover novel TOR controlled biology, this includes the identification of a new TOR inhibitor (Scyl1) required for survival upon stress to endo-membranes and a role for TOR signalling during cytokinesis and cell division.

SYM-33-01
CONTROL OF SHOOT ARCHITECTURE BY STRIGOLACTONE AND KARRIKIN SIGNALLING

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Strigolactone (SL) and karrakin (KAR) signalling pathways play important roles in plant vegetative development, from the commitment of a seed to germinate through to the architecture of the mature shoot. The KAR pathway plays influences germination, seedling photomorphogenesis and leaf shape. The SL pathway influences leaf and stem growth, and controls the outgrowth of lateral shoots and tillers. Perception of SL and KAR signals is achieved by closely-related esterase-type receptors, known in Arabidopsis as DWFAR1 (D14) and KARRIKIN-INSENSITIVE2 (KA12), respectively. Each works in partnership with an F-box protein known as MORE AXILLARY GROWTH 2 (MAX2). Chaperone-type proteins SUPPRESSOR OF MAX2-1 (SMAX1) and SMAX1-Like (SMXL) are downstream targets for ubiquitin-mediated degradation. The biosynthesis of SLs is well understood but the endogenous substrate for KA12 is unknown. Another unknown is the mode of action of SMXL proteins. They are implicated in the control of transcription through interaction with TOPLESS-type proteins, and in the control of auxin transport through the trafficking of auxin efflux carrier (PIN) proteins in the endomembrane system. Both processes are implicated in the control of axillary bud outgrowth to form shoot branches and tillers. A proposed target for SL signalling is the gene BRANCHED 1 (BRC1), but evidence for this is indirect, and BRC1 is not necessary or sufficient to suppress shoot branching. The mode of action of SLs and the interactions of SL, auxin, cytokinin and sugar signalling in the control of shoot branching will be discussed. The implications for the yield of fruits and seeds in crops will also be discussed.

SYM-33-02
SWEET TIMING: ROLES FOR SUGAR SIGNALS IN CIRCADIAN TIME-KEEPING IN ARABIDOPSIS

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The rotation of the planet gives rise to dramatic but predictable fluctuations in light and temperature in terrestrial environments. Circadian clocks have evolved to allow organisms to anticipate these daily and seasonal shifts in the environment. Circadian clocks are set by external cues, through a process called entrainment, to adjust physiology and metabolism to align these rhythmic processes with local conditions. We have recently shown that rhythmic sugar signals, derived from photosynthesis, act as an entrainment cue to adjust circadian time-keeping in Arabidopsis (Haydon et al. Nature 2013). This signalling pathway acts specifically to repress the morning-active clock component PSEUDO RESPONSE REGULATOR 7 (PRR7). We have also identified a distinct role for sugar in sustaining circadian rhythms in the dark that depends on the evening-active clock component CIRCADIAN TIME-KEEPING IN ARABIDOPSIS (CTK1) (Dalchau et al. PNAS 2011). Our most recent data reveal that this requires a GI-interacting F-box protein, ZEITLUPE (ZTL), and an important CONSTITUTIVE TRIPLE RESPONSE 1 (CTR1), a negative regulator of ethylene signalling. We propose a model in which sugar stabilizes the GI-ZTL interaction during the evening, which is diminished after dusk as cellular sugar concentrations decrease through the night to allow ZTL to target the circadian transcriptional repressor, TIMING OF CAB 1 (TOC1), for degradation. The discovery that CTR1 affects circadian rhythms reveals a previously unknown role for ethylene signalling that shares common features with input of sugar signals to the circadian oscillator.

SYM-33-03
REGULATION OF VEGETATIVE-TO-REPRODUCTIVE TRANSITION BY SUGAR METABOLISM AND SIGNALLING

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Vegetative to reproductive transition takes place in the shoot apex meristem (SAM). This phase change represents a key developmental event in plant life cycle and determines yield potential in most crops. Emerging evidence points to the availability of sugars as a key requirement for this process. However, the underlying mechanism remains unclear. We hypothesized that cell wall invertase (CWIN) that hydrolyses sucrose into glucose and fructose in the apoplasm may play a positive role in the transition by facilitating the delivery of sugars as nutrient and signalling molecules to SAM. To investigate this possibility, we examined a set of Arabidopsis single and double mutants for AtCWIN2 and 4, two CWIN genes that are predominately expressed in SAM. Our analyses revealed reduced CWIN activities in the SAM of the atcin mutants, leading to reduced glucose level but increased sucrose level. Importantly, the atcin mutants displayed a significant delay in vegetative-to-reproductive transition and consequently, a late bolting and flowering phenotype. This phenotype corresponds with elevated expression of a floral repressor, miR156, prior to the initiation of the reproductive transition. Interestingly, the expression of SPL9, a transcription factor activating floral initiation, was repressed in the atcin mutants. Together, our data indicate that CWIN positively regulates vegetative-to-reproductive transition, probably through sugar signalling to suppress miR156, thereby allowing SPL9 to be expressed to promote the developmental transition in SAM.
Peptide hormones play important roles in plant development. The identification of peptide hormones in plants and the characterisation of their structure and function is, however, not straightforward. A routine mass spectrometric approach was developed using Medicago hairy root cultures and xylem sap to identify and characterise diverse peptide hormones. We identified 749 spectra corresponding to the in vivo forms of eleven newly identified peptide hormones including four CEP (C-TERMINALLY ENCODED PEPTIDE), two CLE (CLV3/ENDOSPERM SURROUNDING REGION RELATED) and six XAP (XYLEM SAP ASSOCIATED PEPTIDE) peptides. Xylem sap and hairy root cultures also contained evidences for high molecular weight xylem sap proteins. MICEP peptides identified were variously glycosylated and, in some cases, glycosylated. The larger-than-expected fragments provided clues to the maturation and release of MICEP and XAP peptides. Root nodule number was increased by CEP hydroxylation and substitutions at key amino acids but reduced by N-terminal extensions. Homogenously modified mono- and tri-arabinosylated MICEP1 were generated by Fmoc synthesis to probe the effect of glycosylation. Surprisingly, tri-arabinosylation abolished MICEP1 biological activity. The MICLE5 and MICLE17 peptides identified inhibited main root growth and increased lateral root number. MIXAP1a, MIXAP1b and MIXAP5 were identified in root cultures while MIXAP1b, MIXAP1c, MIXAP3 and MIXAP7 were identified in xylem sap. MIXAP1a and XAP5 inhibited lateral root emergence. Transcriptional analyses demonstrated that most peptide hormone genes expressed in the root vasculature. XAP phenotypes revealed five major types of XAPs and MIXAP3 homology to Arabidopsis CASPARIAN STRIP INTEGRITY FACTOR (CIF). Since hairy roots can form on many plants, their corresponding root cultures may be ideal starting materials to identify diverse peptide hormones.

**SYM-33-04**

MASS SPECTROMETRY ANALYSIS OF MEDICAGO TRUNCATULA SECRETOME IDENTIFIES SEVERAL REGULATORY PEPTIDE HORMONE AFFECTING ROOT GROWTH AND DEVELOPMENT

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Recently, we have observed that an abundant aquaporin in Arabidopsis could conduct ions in heterologous systems. This indicates that a subset of plant aquaporins could function as dual water-ion permeable channels. The Arabidopsis Plasma Membrane Intrinsinc Protein AIP2;1, when expressed in Xenopus laevis oocytes, induced an ionic conductance that can be carried in part by sodium ions, and is inhibited by calcium, cadmium and protons. When four PiPs associate to form a tetramer a central channel is created and we hypothesise that the central channel can function as an ion channel. When AIP2;1 expressing oocytes were treated with secondary messengers or kinase inhibitors that alter the activity of native oocyte kinases we observed changes in ionic conductance. This indicates that the ion channel function of AIP2;1 can be regulated by protein phosphorylation. Currently we are testing the physiological role of AIP2;1 in root ion transport. We observed that the roots of AIP2;1 loss of function mutants differ in their response to changes in external salt and osmolality relative to roots of wild type Arabidopsis plants. We are testing the hypothesis that AIP2;1 is a candidate for a non-selective cation channel associated with Na+ and K+ uptake into plant roots and exploring the role of AIP2;1 in root turgor adjustment.
SYM-34-03
IDENTIFICATION OF GENES PLAYING A ROLE IN METABOLISM BY HIGH-THROUGHPUT MOUSE PHENOTYPING IN THE INTERNATIONAL MOUSE PHENOTYPING CONSORTIUM (IMPC)

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Metabolic diseases such as obesity and T2DM cause increasing health problems worldwide. To better understand the relevance of the underlying genetic factors, large-scale research is required in particular to address the role of so far unannotated mammalian genes that may show links to metabolic dysfunction. The International Mouse Phenotyping Consortium (IMPC) aims to produce a knockout mouse line for every protein-coding gene. Highly standardized phenotyping data are generated in phenotyping facilities from Europe, North America, and Asia to compile a comprehensive catalogue of genes related to human disease. Phenotyping data are publicly available at www.mousephenotype.org. Researchers also have access to the mouse models for further studies. To suggest a standardized data analysis procedure to search for genes linked to metabolic disorders, we evaluated metabolic phenotypic data of more than 2,000 knockout strains covering glucose and energy homeostasis, body mass, and lipid metabolism. About half of the gene knockouts caused strong metabolic phenotypes and many of those had not been linked to metabolism before or were functionally unannotated in mice so far. We also evaluated novel links to human disease by searching for SNP’s associated with metabolic disease traits. The results highlight the capacity of IMPC to provide phenotyping data and new mouse models that are useful to identify human disease genes and to direct future in-depth research.

SYM-34-05
VISUALISING THE NONSENSE

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The nonsense mediated decay pathway (NMD) plays an imperative role in normal brain development. Several key pathway members are implicated in many neuro developmental disorders including childhood intellectual disability. Recently NMD activity has been discovered to be variable across cell types and display inter-individual variability. Current methods to quantify NMD activity are end point assays which report on total populations of cells, and thus fail to capture dynamic changes resulting from cellular heterogeneity. To overcome these limitations we have engineered a novel fluorescence reporter transgene which can resolve NMD activity at the single cell level. Our single transgene is comprised of three expression cassettes, namely the selection, control and NMD responsive cassette. The control and NMD responsive cassette co-express distinguishable fluorescent proteins allowing for visual and quantitative real-time output of NMD activity, which are also conducive to standard protein and RNA methods. Using these methods we have shown our NMD reporter transgene to be responsive to NMD inhibition in vitro. The selection cassette utilises the Fip/Frt recombination mediated cassette exchange system allowing the transgene to be stably incorporated into the Col1A1 locus of germ-line competent mouse ES cells. Therefore for the first time this tool allows the creation of NMD reporter mouse lines. This technology can provide visual and quantitative tracking of endogenous NMD activity at a single cell resolution during embryonic brain development and into postnatal life. Furthermore the definition of regions/cell types in the brain most affected by pathogenic NMD disrupting mutations can direct more targeted therapies.

SYM-34-04
METAL BASE IMAGING COMPOUNDS FOR IMAGING ORGANELLES

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Live cell imaging is becoming an important tool in understanding the molecular mechanisms underlying cellular functions. As technology for live cell imaging becomes more readily available there is an increased need and demand for better imaging regents. Fluorescent imaging in live cell can be particularly problematic with many commercially available dyes based on a small number of organic fluorophores which are prone to photo-bleaching, self-quenching, the formation of excimers in cells and can be cytotoxic following prolonged exposure. In addition these reagents are often unstable at room temperature and light sensitive making them difficult to store reliably. The development of metal based luminescent probes is an area of expanding interest as these probes are less sensitive to photo-bleaching, have high emission yield, large Stoke shifts and long emission life-times, making them ideal for a range of fluorescence based applications. We have recently developed a new range of rhenium based probes which are ideal for live cell imaging. In add these probes are stable at room temperature, have low cytotoxicity and are resistant to photo-bleaching making them ideal for live cell imaging experiments. We demonstrate that one of these new rhenium and iridium based probe are taken up by cells freely and can be specifically localised within cells including to the perinuclear region, mitochondria and high lipid content compartments. These new tools have provided cell biologist with greater insight into cell process and a number of important metabolic processes.

SYM-35-01
TRANSCRIPTIONAL TARGETS IN HEART DEVELOPMENT AND OFF-TARGETS IN CONGENITAL HEART DISEASE

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Mutations in core cardiac transcription factors, including NKX2-5, GATA4, TBX5/20, are found commonly in familial cases of congenital heart disease (CHD). We have adapted the technique of DNA adenine methyltransferase identification (DamID), first used in whole Drosophila to identify targets of chromatin-binding proteins, to profile the targets of cardiac transcription factors (TF) in the cardiomyocyte HL1 cell line. DamID is complementary to chromatin immunoprecipitation (ChIP) but avoids artefacts associated with chromatin crosslinking and poor quality ChIP antibodies. Focusing on the targets of WT NKX2-5 and mutant proteins causative for CHD, we made the discovery that mutant proteins, even those lacking DNA binding ability, bind to a subset of nontargets as well as hundreds of unique targets termed “off-targets”. Mutant proteins bind to targets and off-targets via their ability to dimerise with WT NKX2-5 and to broadly or ubiquitously-expressed NKX2-5 cofactors. Different mutants have distinct target and off-target signatures. Altered DNA binding specificity has also been documented. From genome-wide DamID data, machine-learning approaches have allowed us to identify previously unknown directly-interacting NKX2-5 cofactors. In an embryonic stem cell assay, expression of mutant NKX2-5 proteins can affect the expression of off-target genes. Our studies have implications for transcription factor function and the mechanism of CHD. We predict that mutation-specific dominant-negative and gain-of-function effects arising from dysregulation of the hundreds of off-targets will destabilise the cardiac gene regulatory network and contribute to CHD pathophysiology. Genome-wide target data may allow classification of CHD phenotypes and long-term prognosis for CHD patients. This concept is broadly applicable to other diseases caused by TF mutations including cancer.
SYM-35-02
PLURIPOTENT STEM CELL MODELS OF HEART DEVELOPMENT AND DISEASE
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Congenital heart disease is the most common form of birth defect, with a prevalence approaching 1 in 100 children. Similarly, cardiovascular disease is a major cause of illness and death in the Western world and is considered "Australia's most costly disease" requiring an estimated annual expenditure of $5.9 billion. Although the etiologies underlying congenital heart disease and cardiovascular disease differ, the development of new treatments for either condition will be critically dependent on a detailed understanding of how the human heart is formed and how it functions at the cellular and molecular level. Human pluripotent stem cell (hPSC) derived cardiomyocytes are the only tractable platform for illuminating the fine detail of the genetic networks that control human cardiomyocyte cell biology. We have developed a cellular framework to investigate the genetic regulation of human cardiac cell lineage specification. We are now utilizing these reagents and technologies to study heart development using differentiating hPSCs. In addition, we have developed hPSC-based models of a number of cardiovascular diseases including cardiac hypertrophy and pulmonary arterial hypertension.

SYM-35-03
LIVE-IMAGING OF INTRA-MOLECULAR TENSION ACROSS ZEBRAFISH VE-CADHERIN UNCOVERS MECHANICAL CONTRIBUTIONS IN DEVELOPMENT AND DISEASE
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It has long been appreciated that physical forces contribute to vascular development. The quantification of mechanical force and analysis of its conversion into biologically relevant information (mechanotransduction) is inherently challenging. The recent application of FRET-based sensors that report tension through adhesive proteins have greatly aided in vitro studies. To visualize and investigate adhesive forces in the developing vasculature in vivo, we generated a zebrafish VE-cadherin FRET-based Tension Sensor (TS) transgenic strain. We placed VE-cadherin-TS under the control of VE-cadherin’s regulatory elements. This regulatory construction created an endothelial cell junctions allowing live-imaging of protein and junctional dynamics. VE-cadherin-TS protein is functional and capable of rescuing a VE-cadherin mutant allele to adulthood. Furthermore, we show that quantifiable FRET reflects actomyosin dependent tension through VE-cadherin using both ratio-metric FRET and lifetime measurements. Applying this novel tool, we found that arterial maturation during development involves a progressive decrease in tension across VE-cadherin. We continue to show that this morphological and tensile maturation requires Vegfr2 and that the presence of tension increases during development, that tensile changes through the transition from early to late arterial maturation in the developing vasculature.

SYM-35-04
THE ROLE OF THE NEDD4 UBIQUITIN LIGASE DURING LYMPHATIC DEVELOPMENT
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The lymphatic vasculature is a crucial component of the cardiovascular system, with vital roles in tissue homeostasis, immune cell trafficking and the absorption of lipids from the digestive system. Although signalling events important for the development of the blood vasculature system have been thoroughly investigated, less is known about the signalling pathways involved in development of the lymphatic vasculature. Our work has revealed that the ubiquitin ligase Nedd4 is crucial for morphogenesis of the lymphatic vasculature during mouse embryogenesis; Nedd4−/−embryos exhibiting strikingly mis-patterned dermal lymphatic vessels. Furthermore, conditional deletion of Nedd4 from lymphatic endothelial cells using the Prox1Cre mouse line results in aberrant dermal lymphatic vessel patterning, demonstrating a cell autonomous role for Nedd4 in lymphatic development. Ubiquitination has been shown to regulate the function of an array of proteins, including tyrosine kinase receptors of the VEGF receptor family, by controlling their stability, subcellular localisation or degradation. Here, we provide evidence demonstrating that Nedd4 regulates lymphatic vascular development in a cell autonomous manner by controlling the abundance and trafficking of VEGF receptors, leading to reduced signalling in response to VEGF-C. Furthermore, we demonstrate that Nedd4 regulates the remodelling of lymphatic endothelial cell adhesions junctions; in the absence of Nedd4, the failure of junctional remodelling results in decreased capacity of lymphatic endothelial cells to migrate in response to VEGF-C. Current work aims to identify the substrates of Nedd4 responsible for regulating lymphangiogenesis.

SYM-35-05
NOTCH SIGNALLING MEDIATES NEURAL CREST CELL AND SECOND HEART FIELD COMMUNICATION TO ORCHESTRATE CARDIAC OUTFLOW TRACT DEVELOPMENT
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Congenital heart defects affect over 1% of all live births and represent the most common birth defect. Much of the complexity of heart development is underpinned by the interaction of multiple different cell types (second heart field [SHF], neural crest cells [NCCs] and endodermal cells) to orchestrate formation of the fully functional heart. Nedd4+ mouse display heart outflow tract defects reminiscent of those observed in children, including double outlet right ventricle (DORV) and persistent truncus arteriosus (PTA). Given we have previously demonstrated a role for the ubiquitin ligase Ned4D in cranial NCC development (Wisznia, 2013, Dev. Biol. 15:186), we hypothesised that Nedd4D may also play a role in cardiac NCCs, underpinning the outflow tract defects observed. Removal of Nedd4D specifically in NCCs (Wnt1-Cre; Ned4D−/−embryos) resulted in outflow tract defects reminiscent of complete Nedd4D knockout, suggesting a specific role for Nedd4D in NCCs. Interestingly, genetic lineage tracing revealed no deficiency of NCCs within the outflow tract. However, cardiac precursors of the SHF, which sit adjacent to the migrating cardiac NCCs, exhibited premature cardiac myocyte differentiation, leading to the hypothesis that Nedd4D may also contribute to the correct lengthening of the outflow tract. This suggests cardiac NCCs signal to the SHF to maintain these cells in a progenitor state, as well as instruct deployment of these cells into the outflow tract. RNAseq and proteomics analysis of SHF and NCC preparations revealed reduced Notch signalling upon loss of Nedd4D. Removal of Notch ligand activity specifically from NCCs (Wntf1-Cre; Ned4D−/−embryos) phenocopied aspects of the Wntf1-Cre; Ned4D−/− heart defect (pulmonary stenosis). Current work is focussed on dissecting the interaction between NCCs and the SHF; particularly how ligands expressed by NCCs signal to Notch receptors on SHF cells, and how this inter-cellular communication orchestrates correct cardiac outflow tract development.
SYM-36-01

UNIQUE NEUTROPHIL GLYCO-SIGNATURES IN INFLAMMATION AND INFECTION

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The granulated neutrophils are front-line immune cells critical for the innate immune system. Many structure/function aspects of the unique neutrophil glycoproteinome, which shows strong subcellular-specific characteristics, remain unresolved. Enabled by maturing glycomics and glycoproteomics technologies, we have now powerful tools to map the involvement of the intriguing protein N-glycosylation of human neutrophils in inflammation and bacterial infection. We initially discovered a under-reported class of truncated N-glycoproteins, paucimannosidic proteins, in sputum from pathogen-infected human lungs (Venkatakrishnan et al., Glycobiology, 25(1):88, 2015). We then demonstrated that 1) their structures encompass simple monosaccharide compositions i.e. Man, GlcNAc,Fuc, 2) the associated biosynthetic machinery involves malnutrition stage-specific expression of β-hexosaminidases and 3) showed a preferential subcellular location in the azurophilic granules of pulmonary neutrophils (Thaysen-Andersen et al., J Biol Chem 290(14):8789, 2015). Importantly, these inflammation-associated glyco-signatures were found to be present on intact bioactive proteins including cathepsin G and neutrophil elastase, demonstrating that they do not arise from typical lysosomal degradation (Loke et al., J Biol Chem 290(14):8789, 2015). In addition, paucimannosidic proteins were secreted upon P. aeruginosa-based neutrophil activation thereby confirming mobility of the azurophilic granules and suggesting extracellular functions of paucimannosidic proteins. Neutrophil-specific glycan signatures were also directed preferential affinity to mannos-binding lectin and showed bactericidal activities towards virulent P. aeruginosa thus supporting immune-related functions of paucimannosylation in activated neutrophils. Interestingly, isolated paucimannosidic proteins secreted from type V secreting human neutrophil disease-pathogen P. aeruginosa showed reduced protein paucimannosylation relative to age-paired healthy individuals thereby confirming a β-hexosaminidase-driven biosynthesis of paucimannosidic proteins and suggesting that these peculiar glyco-signatures may underpin the pathogenesis of Sandhoff disease. This talk will summarise our growing evidence on the presence and involvement of unique neutrophil glyco-signatures during inflammation and infection.

SYM-36-02

DISSECTING THE SUBCELLULAR SECRETORY GLYCOPROTEOME

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N-glycosylation is a critical post-translational modification that influences the folding and function of half of the cellular proteome. The biosynthesis of N-glycoproteins begins in the endoplasmic reticulum (ER), where an oligosaccharide is transferred to selected asparagine residues in nascent polypeptides by the enzyme oligosaccharyltransferase. The presence of N-glycans at specific sites is critical for efficient productive protein folding in the ER, and defects in this process perturb glycoprotein folding, secretion, and function at a systems level. We have developed integrated subcellular fractionation and SWATH glycoproteomic workflows to understand the causes and consequences of changes in the N-glycosylation biosynthetic pathway. We combined biochemical subcellular fractionation methods with quantitative SWATH-MS glycoproteomic and proteomic workflows to measure the response to a range of genetic and chemical perturbations to N-glycoprotein biosynthesis. We optimized biochemical fractionation methods in yeast to enable precise analysis of the subcellular proteome and glycoproteome. This enabled quantitative measurement of subcellular proteomes and site-specific and global profiling of glycan occupancy and structure. We identified known and novel glycoproteins with defined defects in N-glycosylation, and then expanded our analysis to profile the quantitative effects of combined defects in glycoprotein biosynthesis and protein quality control on glycoprotein maturation. Our results give key insights into the effect of site-specific glycosylation on glycoprotein quality control processes, and our methods will be useful in diverse applications in industrial and medical glyco-biotechnology.

SYM-36-03

CELL WALL CARBOHYDRATE STRUCTURE AND BIOSYNTHESIS IN PATHOGENIC OOMYCETES

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The oomycete phylum comprises devastating pathogens of crops and animals that represent a serious threat to food production and sustainability. Their cell walls share structural features with both plants and fungi. Like plant cells, oomycete hyphae contain cellulose as the main load-bearing component, whereas chitin, a typical major cell wall component of fungi, occurs in minute amounts in the walls of some oomycete species only. Similar to fungal cell walls, oomycetes produce a diversity of β-glucans that consist essentially of β-(1,3) and β-(1,6) glucosidic linkages. Thus, oomycetes represent interesting model systems for cellulose biosynthesis in plants and β-glucan and chitin biosynthesis in fungi. In addition, the enzymes responsible for cell wall biosynthesis in oomycetes represent potential targets of inhibitors that can be used to control the diseases provoked by pathogenic species. However, the proteins associated to the oomycete carbohydrate synthase complexes and their corresponding mannoprotein-glycosyltransferases that form cellulose and chitin in two devastating pathogens, Phytophthora capsici and Phytophthora infestans, which infects a large number of crops, and the fish parasite Saprolegnia parasitica. Different recombinant forms of the enzymes were expressed in heterologous systems and characterised in vitro, providing insight into the molecular mechanisms and structural organisation of chitin and cellulose synthases.

SYM-36-04

NEW INSIGHTS INTO THE REGULATION AND INHIBITION OF BACTERIAL AGGREGATION/BIOFILM FORMATION

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The self-associated autotransporters (SAATs) are a group of glycosylated proteins from widespread E. coli pathogens that are transported to the cell surface by the type V secretion pathway. These virulence factors have central roles in bacterial aggregation and biofilm formation, which are important attributes for colonisation and persistence, which is extremely relevant to the areas of bacterial pathogenesis, noncommensal infections and food sanitation. We recently elucidated the mechanism by which the SAAT Antigen43 from uropathogenic E. coli (UPEC) promotes bacterial aggregation/biofilm formation, by means of self-association between neighbouring cells. We sought to determine if all SAATs shared a common mechanism for facilitating bacterial aggregation/biofilm formation, if this function was regulated and if it could be inhibited. The SAAT TiaB from the highly virulent enterotoxigenic E. coli (ETEC) was known to be glycosylated by the Tmb glycosyltransferase. We determined the crystal structures of the glycosylated and unglycosylated forms of TiaB and used this to inform further biochemical and phenotypic studies. We found that TiaB self-associates similarly to that of Antigen43, but with a more extensive interface, to facilitate bacterial aggregation/biofilm formation. Glycosylation by Tmb was found to physically block TiaA that TibA self-associates similarly to that of Antigen43, but with a more extensive interface, to facilitate bacterial aggregation/biofilm formation, if this function was regulated and if it could be inhibited. The SAAT TiaB from the highly virulent enterotoxigenic E. coli (ETEC) was known to be glycosylated by the Tmb glycosyltransferase. We determined the crystal structures of the glycosylated and unglycosylated forms of TiaB and used this to inform further biochemical and phenotypic studies. We found that TiaB self-associates similarly to that of Antigen43, but with a more extensive interface, to facilitate bacterial aggregation/biofilm formation. Glycosylation by Tmb was found to physically block TiaA self-associates similarly to that of Antigen43, but with a more extensive interface, to facilitate bacterial aggregation/biofilm formation.
SYM-36-05
TREHALOSE-6-PHOSPHATE PHOSPHATASE: AN EMERGING TARGET FOR PARASITIC DISEASE THERAPIES
Cross M.O.1, Rajan S.1, Kim J.S.2, Gasser R.B.3 and Hofmann A.3, 4
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Over 400 million people are currently affected by parasitic infections. While not associated with high mortality rates, parasitic worm diseases are extremely debilitating and carry significant long-term consequences with current global burden estimated at 22 million disability adjusted life years (DALYs) per annum. The widespread occurrence of parasitic infections in both humans and livestock further imposes a substantial socio-economic burden that grows heavier as climate change and global expansion make more environments amenable to parasite transmission. Increasing reports of spread and drug resistance have thus prompted a call for new strategies for prevention and treatment. Trehalose is an essential disaccharide in many pathogens, but is neither required nor synthesised in mammalian hosts. As such, trehalose-6-phosphate phosphatase (TPP), a key enzyme in trehalose biosynthesis, is an attractive target for novel chemotherapeutics. Here, as first steps in the drug discovery pipeline, we investigate the structural basis of TPP substrate specificity and identify key residues for the enzymatic mechanism through a molecular-dynamics-informed mutagenesis study. Through structure-based sequence analysis of a panel of TPP enzymes from bacterial and nematode pathogens, we propose their classification into three distinct topological groups. Finally, purification and enzymatic characterisation of TPPs from five important nematode and bacterial pathogens reveals that all five enzymes display burst-like kinetic behaviour which is characterised by a decrease in the enzymatic rate after the pre-steady state. The observed super-stoichiometric burst amplitudes can be explained by global conformational changes by members of this enzyme family during substrate processing. The findings from this study suggest complex conformational transitions in TPPs during the catalytic cycle and provide a foundation for rational inhibitor design in future TPP structure-based drug discovery work.

SYM-37-01
INTERGENIC DISEASE-ASSOCIATED REGIONS ARE ABUNDANT IN NOVEL REGULATORY TRANSCRIPTS
Bartonicek N.1, Clark M.B.2, Quek X.C.1, Torpy J.3, Pritchard A.L.4, Hayward N.K.1, Montgomery G.W.1, Mat trick J.S.4, Merc er T.R.4 and Dinger M.E.5
1Garvan Institute of Medical Research, Sydney, New South Wales, Australia. 2Department of Psychiatry, University of Oxford, Warneford Hospital, Oxford, United Kingdom. 3QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia.
Genotyping of large populations through genome-wide association studies (GWAS) has successfully identified many genomic variants associated with traits or disease risk. Unexpectedly, a large proportion of GWAS SNPs and associated haplotype blocks are in intronic and intergenic regions, hindering their functional evaluation. While some of these risk-susceptibility regions encompass cis-regulatory sites, their transcriptional potential has never been systematically explored. To detect rare tissue-specific expression, we employed the transcript enrichment method CaptureSeq on 21 human tissues to identify 1775 multi-exonic transcripts from 581 intronic and intergenic haplblocks associated with 392 traits and diseases, covering 73.9 Mb (2.2%) of the human genome. We show that a large proportion (85%) of disease-associated haplblocks express novel multi-exonic, non-coding transcripts that are tissue-specific and enriched for GWAS SNPs as well as epigenetic markers of active transcription and enhancer activity. Similarly, we captured transcriptomes from 13 melanomas, targeting 9 melanoma-associated haplblocks, and characterized 31 novel melanoma-specific transcripts that include fusion proteins, novel exons and non-coding RNAs, a third of which showed allelically imbalanced expression. This resource of previously unreported transcripts in disease-associated regions (http://capseq-dev.dingerlab.org) should provide an important starting point for the translational community in search of novel biomarkers, disease mechanisms and drug targets.

SYM-37-02
ANALYSING AND VISUALISING SINGLE-CELL RNA-SEQ DATA
Oshlack A., Pipson B. and Zappia L.
Murdoch Children’s Research Institute, Parkville, Vic 3052, Australia.
Single-cell RNA sequencing (scRNA-seq) is rapidly becoming a tool of choice for biologists wishing to investigate gene expression at greater resolution, particularly in areas such as development and differentiation. The minute amounts of RNA in each cell need to be amplified to a great extent before sequencing. This process often results in noisy data with lots of technical artefacts. Therefore single-cell data presents an array of bioinformatics challenges. In this talk I will take a bioinformatician’s view of single-cell RNA-seq analysis. I will use examples of our experience using single-cell data to analyse kidney development in mouse and kidney organoids. This will provide insights into how future single-cell experiments should be designed and analysed.

SYM-37-03
GLIMMA: GETTING GREATER GRAPHICS FOR YOUR GENES
Su S., Law C.W. and Ritchie M.E.
Molecular Medicine Division, The Walter and Eliza Hall Institute of Medical Research.
RNA-sequencing is a popular technology for studying changes in gene expression across tens of thousands of transcripts simultaneously. To make exploration of gene expression data easier, we developed Glimma, an R package which generates interactive plots for gene expression analyses. Glimma plots connect the many layers of information in a single html page using d3.js. For example, a Glimma-style mean difference plot, allows one to select a point from a display of summary statistics to reveal the sample-wise expression levels alongside the original plot. This feature enables researchers to interrogate the data more easily by allowing searches for genes or samples of interest and zooming for better resolution. Unlike the traditional multi-dimensional scaling (MDS) plot, Glimma’s MDS plot shows several dimensions and group combinations on the same page. Results from Glimma can be easily shared between bioinformaticians and biologists, enhancing reporting capabilities while maintaining reproducibility. Besides bulk RNA-sequencing data, Glimma can also handle data from microarray, single-cell RNA-sequencing and methylation experiments.
**SYM-37-04**

**SELF-GENERATING AUTO-REGULATORY SIGNALS MODIFY PROTEIN FUNCTION IN INNATE IMMUNITY**

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Monash Institute of Pharmaceutical Sciences, Monash University, Parkville VIC 3052.

Using system biology approaches, we have identified a subset of moonlighting proteins that contain ‘catalytic’ centres which act to alter the microenvironment surrounding the protein. Our archetypical example is a guanylate cyclase (GC) catalytic centre embedded within the kinase domain. For instance, in the phytosulfokine receptor (PSKR1), kinase and GC activity are reciprocally regulated by changes in calcium and phosphorylation while cGMP inhibits kinase activity. Cyclic GMP has similar effects on several other proteins containing a GC centre. These observations led us to consider that accumulation of signal products within the protein microenvironment may have an auto-regulatory function. This concept was enhanced by our identification of another GC centre that is active in regulating the mammalian innate immune system, IRAK3 (interleukin 1 receptor associate kinase 3). When the GC centre is inactivated, IRAK3 no longer inhibits NFκB induced gene transcription, but this ability can be recovered in the presence of subnanomolar levels of membrane permeable cGMP. We are now testing the role(s) in auto-regulation of the immediate microenvironment of the protein. Importantly, such effects can be contained within the microenvironment of protein complexes thereby keeping reactions to micro-cues separated from major cellular responses. Our findings will add to understanding of micro-regulation of protein function and also how spatial and temporal positioning influences cellular (and potentially bodily) function in inflammatory and other disease states.

**SYM-37-05**

**DIRECT TRANSCRIPTIONAL REGULATION BY MICRONRNAS: THE “DARK MATTER” OF MICRONRNA FUNCTION**

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Centre for Cancer Biology, University of South Australia and SA Pathology, Frome Rd, Adelaide, South Australia, 5000.

MicroRNAs are almost always regarded as cytoplasmic, negative post-transcriptional regulators of gene expression. Recent studies however reveal an abundance of mature miRNAs (and accessory proteins) within the nucleus and several reports now postulate direct gene regulatory roles for miRNAs (both positive and negative) at the level of transcription. We have strong evidence that the contribution of these nuclear roles to miRNA activity has thus far been vastly under-appreciated. We have published extensively upon the role of the miR-200 family as a regulator of Epithelial-Mesenchymal Transition (EMT), a reversible phenotypic switch that underlies both normal physiology (such as in embryonic development and wound healing) and cancer progression (facilitating metastasis and altering drug sensitivity). In addition to its well-characterised post-transcriptional repression of EMT-associated genes within the cytoplasm, we also observe miR-200 binding sites are present within gene promoters that are strongly associated with transcriptional regulation. Further, we find that endogenous miRNA:Argonaute (AGO) complexes are present proximal to the transcription start sites of many genes. As miRNAs regulate ALL cellular processes (including EMT), establishing and accounting for these nuclear functions is imperative.

**SYM-38-01**

**RE)DEFINING THE MOLECULAR CONTROL OF APOPTOSIS**

Chin H.S.1,2, Van Delft M.1,2, Li M.X.1,2, Huang D.C.S.1,2 and Dewson G.1,2
1 Walter and Eliza Hall Institute, Melbourne, Victoria, Australia. 2 University of Melbourne, Dept Medical Biology, Melbourne, Victoria, Australia.

BAX and BAK are the essential effector proteins that promote mitochondrial outer membrane permeabilisation (MOMP) and cell death during apoptosis. Understanding the protein interactions that control their deadly apoptotic activity will identify new ways to target apoptosis therapeutically. VDAC2 is thought to be a critical inhibitor of the pro-apoptotic protein BAK. Our studies challenge this dogma. Our data indicate that VDAC2 is important for efficient mitochondrial localisation of both BAX and BAK and is an essential promoter of BAX apoptotic function. Cells lacking VDAC2 and BAK are largely resistant to apoptosis induced by diverse stimuli including BH3-mimetics and deletion of VDAC2 alone was sufficient to protect cells from BAX-dependent apoptosis both in vitro and in vivo. Moreover, deletion of VDAC2 accelerates MYC-driven AML confirming that VDAC2 is also a key mediator of BAX apoptotic function in the context of oncogenic stress as well as chemotherapy. Thus, disrupting the interaction of BAX with VDAC2 is a potential mechanism of chemoresistance or to impair unwanted or damaging apoptosis for example following ischemic stroke or reperfusion injury.

**SYM-38-02**

**DISEASE MODELS OF MITOCHONDRIAL DYSFUNCTION**

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Harry Perkins Institute of Medical Research and School of Molecular Sciences, The University of Western Australia, Nedlands WA 6009, Australia.

The size and organization of the animal mitochondrial genome has been reduced and compacted significantly since its endosymbiosis from an α-proteobacterial ancestor. This compaction has necessitated the evolution of unique mechanisms to facilitate rapid changes in gene expression in response to the changing energy demands of the cell. The mitochondrial transcriptome encodes proteins that are subunits of the respiratory chain, responsible for most of the energy production required by the cell. Consequently the coordinated regulation of the mitochondrial transcriptome by the nucleus is of particular importance for the maintenance of cell health and energy metabolism. We have been investigating the unusual features of mitochondrial RNAs and the RNA-binding proteins that control their production, maturation, translation and stabilization to understand the regulation of mitochondrial gene expression and its contribution to health and disease. I will highlight the devastating consequences of dysregulated mitochondrial gene expression in different models of disease caused by genetic disruption of RNA-binding proteins. Mouse models of disease have enabled us to understand the in vivo role of fundamental processes that regulate mitochondrial RNA metabolism and the pathogenesis of diseases caused by impaired gene expression.
Mitochondria are crucial players in cell metabolism, which requires tight regulation of ion exchange between the cytosol and mitochondrial matrix. This exchange is mediated by a class of multi-membrane spanning proteins localised to the mitochondrial inner membrane, known as the mitochondrial carrier proteins. Mitochondrial carrier proteins are inserted into the inner membrane by a protein import complex known as the Translocase of the Inner Membrane 22 (TIM22 complex). Despite the tight conservation of protein import subunits from yeast to humans, the human TIM22 complex has a distinct subunit composition to its yeast counterparts. In this study, we discovered the presence of a novel subunit, Acylglycerol kinase (AGK), which was previously described to be a mitochondrial lipid kinase. Mutations in AGK cause Sengers syndrome, a mitochondrial disorder characterised by lactic acidosis, hypothyroid cardiomyopathy, skeletal myopathy and congenital cataracts. We generated a CRISPR knock-out of AGK and established that the protein has a kinase-independent function at the TIM22 complex, where it mediates the assembly of the TIM22 complex and the import of mitochondrial carrier proteins. We observed similar protein import defects and destabilisation of the TIM22 complex in mitochondria isolated from Sengers syndrome patient cells. This data uncovers the important and unexpected relationship between the TIM22 complex, mitochondrial protein import and Sengers syndrome.

Mitochondrial electron transport chain complexes are organized into supercomplexes responsible for carrying out cellular respiration. Recently we determined the architectures of mammalian (ovine) supercomplexes (SCs) determined by cryo-electron microscopy. We identified two distinct arrangements of SC I+II+III+IV (the respirasome): a major "tight" form and a minor "loose" form (resolved at the resolution of 5.8 Å and 6.7 Å, respectively), which may represent different stages in supercomplex assembly or disassembly. All observed density can be attributed to the known 80 subunits of the individual complexes, including 132 transmembrane helices. The individual complexes form tight interactions that vary between the architectures, with complex IV subunit COX7a switching contact from complex III to complex I. More recently, via a novel preparation the structure of isolated functional SC I+II+III+IV, has been determined to ~4.0 Å resolution. This structure allows for the detailed analysis of contacts between the complexes, which have clearly evolved to form specific interactions.

This makes it a potential target for improving tolerance to multiple abiotic stresses simultaneously. The alternative pathway consists of a number of type II NAD(P)H dehydrogenases (NDs) and alternative oxidases (AOX). A large research effort has highlighted AOX as a key player in the response to numerous environmental stresses in some plant species. Less is known of the NDs, but they are also hypothesised to play a role in plant stress responses. In Arabidopsis thaliana the transcript level of NDB2 (an ND isoform) is typically co-expressed with the most stress-responsive AOX gene, AOX1A, and both are responsive to a range of stresses. Here we use a reverse genetics approach to demonstrate that (i) NDB2 can work together with AOX1A to provide a fully functional alternative pathway of respiration in plant mitochondria, and (ii) NDB2 and AOX1A are both important for dealing with photo-inhibitory stress. Specifically, plants lacking either the AOX1A or NDB2 protein were more sensitive to combined drought and elevated light treatments, while plants overexpressing these components showed increased tolerance and capacity for post-stress recovery. These results align with the emerging hypothesis that the alternative pathway helps prevent photosynthesis during environmental stress, and we suggest that both AOX and NDs are important for this.

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SYM-39-02
REVERSING VASCULAR AGEING USING NAD+/-H2S SIGNALING NETWORK

Das A.1,2,3, Huang G.2, Bonkowski M.2, Schultz M.2, Hung T.1, Wu L.E.1, Turner N.1, Arany Z.P.2, Guarente L.P.3 and Sinclair D.A.1
1University of New South Wales, 2Harvard Medical School, 3Massachusetts Institute of Technology, 4University of Pennsylvania.

In skeletal muscle, endothelial cell dysfunction, impaired microcapillary formation, and a progressive decline in exercise capacity are hallmarks of aging, yet the underlying causes are poorly understood. Sirt1, a NAD+-dependent protein deacetylase, is a pro-longevity factor required for many of the health benefits of caloric restriction. Recent studies have identified Sirt1, a NAD+-dependent protein deacetylase, as a critical regulator of endothelial homeostasis, where it promotes angiogenesis, and inhibits senescence and apoptosis. Metabolic precursors of NAD+ have attracted attention for their ability to reverse the decline in NAD+ levels during aging, and activate Sirt1. Here, we show that deletion of the Sirt1 gene specifically in endothelial cells results in an accelerated loss of capillary density and exercise capacity during aging, along with an inability to respond to exercise. Conversely, overexpression of Sirt1 in endothelial cells increases capillary density and maintains the endurance capacity of aged mice. Together, these studies demonstrate that precursors of NAD+ have the potential to reverse the effects of aging in endothelial cells and, therefore, might be useful in the treatment of muscle degenerative diseases such as DMD.

SYM-39-03
CALORIES AND MACRONUTRIENTS IN LATELIFE HEALTH AND AGEING

Solon-Biet S.M.1,2, Cogger V.C.1,2, Pulpel T.1,2, Wahl D.1,2, Le Couteur D.G.1,2 and Simpson S.J.1,2
1Charles Perkins Centre, University of Sydney, 2Centre for Education and Research on Ageing and the ANZAC Research Institute Concord Hospital, University of Sydney.

The balance of dietary macronutrients has profound effects on health and lifespan. Under ad libitum feeding conditions, caloric restriction by dilution did not extend lifespan in mice. Rather, diets low in protein and high in carbohydrate (LPHC) improved cardiometabolic health, immune function and increased longevity. Animals on LPHC diets had the lowest levels mTOR activation, a key regulator involved in cell proliferation, survival and protein synthesis. Decreased activation was closely linked to reduced circulating branched chain amino acids (BCAA) and glucose, suggesting that ad libitum LPHC diets may be the key to delaying ageing and age-related disease. When we compared diets varying in protein to carbohydrate ratio under both 40% caloric restriction and ad libitum conditions over 8 weeks, we found that ad libitum LPHC diets delivered similar benefits to CR in terms of levels of insulin, glucose, lipids and HOMA, despite increased energy intake. CR on LPHC diets did not provide additional benefits relative to ad libitum LPHC and show that LPHC diets under ad libitum-fed conditions generate the metabolic benefits of CR without a 40% reduction in total caloric intake. A central priority is to further investigate and compare the long-term effects of CR and ad libitum LPHC diets on metabolic health and lifespan in mice, as well as to begin to consider the effects of the type and quality of proteins. Here, we evaluate the effects of ad libitum LPHC, 40% CR and BCAA supplementation on health and longevity in 960 mice. The ultimate aim is to explore the relationship between nutrition, metabolic health and the ageing process. These findings may have important implications for diet management in laterlife metabolic health and ageing.

SYM-39-04
WNT-ß-CATENIN SIGNALLING REGULATES MUSCLE REGENERATION AND FIBROSIS BY DIVERGENT MECHANISMS

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1Department of Clinical Pharmacology, Flinders University, South Australia 5042, 2University of California, San Francisco, 3School of Medical Sciences, University of New South Wales.

Canonical Wnt signalling regulates muscle stem cell/myoblast differentiation, but there have been conflicting reports about the requirement for ß-catenin in adult regenerative myogenesis. ß-catenin is also known to be a key player in fibrosis in many tissues. We proposed that Wnt may be a double edged sword in muscle repair, both promoting myogenesis but also contributing to pathogenic fibrosis. To better understand the role of Wnt in myogenesis, we used CRISPR to generate ß-catenin null primary adult mouse myoblasts in vitro. ß-catenin null myoblasts showed greatly impaired spontaneous- and Wnt3a-induced differentiation. RNAseq showed a strong delay in activation of the global myogenic differentiation program after Wnt treatment confirming the requirement for ß-catenin in myogenesis. ß-catenin interacts with TCF/LEF factors but also with the muscle regulatory factor MyoD, and it was unclear which regulatory complex may be involved in myogenesis. Using ChiPseq analysis we showed that Wnt induced activating histone modifications at genomic regions that contain MyoD (E-box) binding elements, but not TCF/LEF elements. We also found that Wnt increased binding of MyoD to E-box elements in myoblasts in wildtype but not ß-catenin null cells. Moreover, a variant of ß-catenin that cannot interact with TCF/LEF resulted in the differentiation capacity of ß-catenin null myoblasts as effectively as wildtype ß-catenin. Together these data indicate that Wnt promotes adult myogenesis in ß-catenin-dependent, but TCF/LEF-independent manner. ß-catenin-TCF/LEF-CBP complexes are known to be involved in fibrosis in many contexts. Because we found no requirement for ß-catenin-TCF/LEF complexes in myogenesis, we postulated that inhibition of this complex would not impair myogenesis but might reduce fibrosis mediated by muscle fibroblasts/FAPs. In support of this, preliminary evidence suggests that a small molecule inhibitor of ß-catenin-TCF/LEF-CBP complexes inhibits fibrosis in vitro and in vivo without inhibiting myogenesis. This may provide an avenue to develop new therapies for muscle fibrosis associated with muscle degenerative diseases such as DMD.

SYM-39-05
THE GOLGI RIBBON: A NEW HUB FOR THE REGULATION OF MTOR SIGNALING AND AUTOPHAGY

Gosavi P., Houghton F. and Gleeson P.A.
Bio21 Institute of Molecular Science and Biotechnology, University of Melbourne.

The Golgi apparatus (GA) is well known to function in transport, processing and sorting of proteins to their appropriate cellular destinations. In addition, recent advances provide evidence that the Golgi contributes to range of other functions such as cell polarization, stress responses, metabolism, and autophagy. The structural organization of the GA varies among different cell types and species. In vertebrates, individual Golgi stacks are fused into a ribbon structure, typically found in a juxtanuclear location in interphase cells. The classic functions of the Golgi, namely membrane transport and glycosylation, do not require a ribbon structure and the relevance of the Golgi ribbon structure has been elusive. We have developed a cell-based system to explore the biological functions of the Golgi ribbon wherein we demonstrate that the membrane tether of the trans-Golgi network, GCC88, regulates the balance between Golgi mini-stacks and the Golgi ribbon. Loss of Golgi ribbon in stable cells over expressing GCC88 resulted in compromised mTOR signaling and a dramatic increase LC3-II-positive autophagosomes. Loss of the Golgi ribbon has been reported in a range of neurodegenerative diseases and here we show using a tau-based in vitro disease model for neurodegeneration that fragmentation of the Golgi ribbon results in a reduction of Golgi-localized mTOR. Lysosomes are considered as the main localization and activation site for mTOR. Our results have uncovered a key role for the ribbon structure of the Golgi as a major cellular site for mTOR signaling and in regulating autophagy, and the dysfunction of this Golgi-localized signaling pathway is relevant in neurodegenerative diseases.
**SYM-40-01**

APPLYING OMICS TO ADVANCE HORTICULTURAL CROP SELECTION - GUAVA WILT RESISTANCE CASE STUDY

Severn-Ellis A.A.1, Bayer P.1, Batley J.1, Edwards D.1, Rees D.J.G.2 and Heazlewood J.L.1

1. University of Western Australia, School of Biological Sciences, 35 Stirling Highway, Crawley, WA, 6009, Australia. 2. Agricultural Research Council, Private Bag X5 Onderstepoort, Pretoria, South Africa 0110.

Guava wilt, caused by the fungus *Nalanthamala psidii* has severely impacted the cultivation of guava in South Africa, Taiwan, Malaysia and Thailand. The lack of control measures and resistant cultivars has left farmers with little choice but to turn to alternative crops. Subsequent emergence of additional *N. psidii* races has furthermore emphasised the necessity for the development of a variety of resistant guava selections to ensure sustainable disease resistance in the long term. Increased accessibility to omics tools has however created the opportunity to generate valuable genomic and transcriptomic information for the non-model crop - pathogen and their interaction. It is anticipated that the insight created by the current study will contribute significantly towards the advancement of guava breeding programs and successful deployment of resistant selections, exemplifying one of the many applications of omics in today’s research arena.

**SYM-40-02**

SPATIALLY RESOLVED ‘OMICs TO IDENTIFY NovEL SALINITY TOLERANCE MECHANISMS IN BARLEY ROOTS

Ho W.H.1, Hill C.B.2, Natera S.2, Lopez D.L.S.2, Yu D.1, Boughton B.A.3, Rupasinghe T.1 and Roessner U.1,3

1. School of BioSciences, The University of Melbourne, 3010 Victoria, Australia. 2. Murdoch University, Perth, Australia. 3. Metabolomics Australia, School of BioSciences, The University of Melbourne, 3010 Victoria, Australia.

Barley (Hordeum vulgare L.) is an essential food and brewing crop and suffers substantial yield loss under saline conditions. Little is currently understood of salinity perception and responses in plant roots, which involve complex changes at the physiological, metabolic, molecular, transcriptional, and genetic level. We develop new tools to unravel how plants respond to the perception of salt stress. Evidence is accumulating that lipid signalling is an integral part of complex regulatory networks involved salinity responses through modifications of membrane lipids, which occur through the activity of phospholipases, lipid kinases and phosphatases that produce different classes of lipid and lipid-derived messengers. These provide spatial and temporal regulatory functions crucial for cell survival, growth and for an appropriate response of the plant to environmental stimuli. Initial analyses indicate that different tissue types within the root respond differently to salt stress in tolerant and sensitive cultivars. Here we study the root responses to salinity using a combination of next generation RNA-sequencing and targeted metabolite and lipid analyses of three key sections of barley roots. We are also using modern lipidomics technologies to compare the root plasma membrane (PM) compositions of different barley genotypes with contrasting salinity tolerance levels upon salt stress. In addition, we are using MALDI-FT-MS based imaging technologies to monitor spatial distributions of metabolites and lipids across root sections of salt-treated tolerant and sensitive barley genotypes. Transcriptomics results are now being integrated with spatial biochemical data, enhancing our understanding of system-wide and tissue-specific responses of roots to salinity stress. Given the lack of fundamental knowledge of the genes and proteins involved in signalling and lipid metabolism under salinity stress, and the enormous potential for biotechnological application in this area, our results provide insight into novel mechanisms responsible for salt tolerance of barley.

**SYM-40-03**

IDENTIFICATION OF THE GOLGI LOCALIZED UDP-GLCNAC TRANSPORTER AND ITS ROLE IN ENDOMEMBRANE LIPID AND PROTEIN GLYCOSYLATION

Heazlewood J.L.

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Glycosylation reactions require activated glycosyl donors in the form of nucleotide sugars to drive processes such as post-translational modifications and polysaccharide biosynthesis. Many of these reactions often occur in the endomembrane using cytosolic-derived nucleotide sugars, which are actively transported into the lumen by nucleotide sugar transporters (NSTs). We recently identified a plant UDP-GlcNAc transporter responsible for the delivery of substrate for the maturation of N-glycans and sphingolipids within the endomembrane. To determine the biochemical phenotype of the UDP-GlcNAc transporter loss-of-function mutants, we have applied both proteomic and metabolomic approaches. Initially we developed a reliable N-glycopeptide enrichment and mass spectrometry-based analytical workflow to detect, identify and quantify N-glycopeptides. Next, we applied lipidomic approaches to profile sphingolipids from loss-of-function mutants. Analysis of omic data indicated that that N-glycopeptides containing complex N-glycans (e.g. GlcNAc) only comprise about 5% of the N-glycopeptide population in mutant lines. In contrast, N-glycans from wild-type plants are comprised of around 35% complex-type N-glycans i.e. those containing GlcNAc. While sphingolipid analysis indicated that GlcNAc containing lipids comprised less than 10% of that observed in wild type plants. Our findings indicate that the reference plant Arabidopsis contains a single UDP-GlcNAc transporter responsible for the maturation of complex N-glycans and sphingolipids in the Golgi lumen. The work also highlights the detailed insight and resolution that can be achieved using modern omic approaches.

**SYM-40-04**

EXPLORING AND IMPROVING GENOME EDITING IN MODEL PLANT NICOTIANA BENTHAMIANA

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CRISPR-Cas9 driven genome editing of crops is rapidly advancing agricultural biotechnology and basic research. The process relies on DNA double stranded breaks at user defined genomic loci and repair by non-homologous end joining (NHEJ) and/or homologous recombination (HR). The endogenous DNA repair mechanisms are predominantly used to knock out genes by NHEJ pathway and knock in genes by harnessing the HR pathway. In plants however, causing knock out mutations by NHEJ is much more efficient than insertion of new sequences using HR. Therefore, in plants there is an opportunity and a need to improve ways of precisely inserting nucleic acid sequences into the genome. We have investigated the use of different Australian viruses and manipulating DNA repair pathways to enhance the technique. Here we report our exploration of the techniques and progress in precisely editing the genome of our model plant, Nicotiana benthamiana.
SYM-40-05
TRANSCRIPTOMIC CHANGES IN ARABIDOPSIS LEAVES INDICATE CAUSES FOR LOSS OF STRESS TOLERANCE WITH AGE
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Ageing as a natural process of plant development is provoked by both, internal and external factors and results in the onset of the final phase of plant life known as senescence. The progressive changes that occur during the time course of growth and development in plants are termed as age-related changes (ARCs). It has been reported by multiple studies that the tolerance of stress decreases with age, however, the underlying molecular mechanisms are not well known. This research, therefore, attempts to better understand the pathological pathways that are involved in the reduction of plant stress tolerance with age, using Arabidopsis thaliana as model. First, we confirmed that drought, salinity and dark stress treatments conducted on Arabidopsis young, mature and adult plants showed increased stress susceptibility with age, consistent with a role for ARCs in stress resistance. Next, the transcriptomes of ageing but not senescing individual rosette leaves were compared by RNA-seq. The analysis of differentially expressed genes showed that leaf maturation coincides with a marked downregulation of genes involved in DNA repair, while genes involved in stress hormone biosynthesis and signalling and genes indicative of oxidative stress were upregulated. This study suggests that young plants are more tolerant to stress because of negligible ARCs in young leaves, whereas the gradual and rapid accumulation of ARCs in mature and old leaves result in decreased tolerance to stress. Moreover, reduction of DNA damage protection and decreased stress tolerance appear to be intrinsically coupled to ageing ensuring a timely and certain death.

SYM-41-01
TERROIR OR NOT TERROIR – THIS IS A DIFFICULT QUESTION!
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The terroir concept is generally applied to agricultural commodities inferring that unique composition, taste and flavour attributes in a product can be achieved only from a specific geographical location. The very notion of how landscapes, people, processes and environmental factors interact is at the heart of terroir. Understanding the interactions of environmental and vineyard management influences on grape berry composition are the mainstay challenges for viticulturists and winemakers worldwide. Grape composition results from numerous interactions between cultivar growing conditions, water availability and the level of berry ripeness. A temporal and spatial investigation of berry, and subsequent wine composition and style was undertaken for two consecutive vintages in climatically diverse regions of Australia. Controlled fermentations of Shiraz and Cabernet Sauvignon grapes from harvest dates were determined at defined berry maturities using a sugar accumulation model to target three wine styles (Fresh, Lush, Mature and Mature). Sensory descriptive analysis of wines enabled different styles to be readily identified. Comprehensive profiling of grape composition, winemaking inputs, wine chemical and volatile composition, in addition to wine sensory scores generated nine individual data blocks for each wine. A metabolomics approach (ANOVA Multiblock Orthogonal PLS) was used to elucidate the impact of experimental factors (vineyard, region, vintage and grape harvest stage) for each cultivar. Loadings extracted from models with significant effects were subject to hierarchical cluster analysis (HCA) following rotation of model components to a consistent direction of effects levels in scores plots. The contribution of each data block to experimental factors, ANOVA designs and HCA assist in understanding the impact of grape berry ripening on Shiraz and Cabernet Sauvignon wine composition. Whilst vintage is a significant contributing factor, differences arising from grape ripening stage, region and vineyard management practices on grape berry and wine compositions could be determined. This approach has provided some clues to the various factors that are important in defining uniqueness among Australian wine regions.

SYM-41-02
GRAPEVINE CANOPY MANAGEMENT USING THE VITICANOPY APP
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Grapevine canopy management involves numerous techniques applied to alter the amount and distribution of shoots, leaves and fruit to obtain a desirable yield/quality ratio. There are several techniques that can be applied to assess canopy architecture and to gather information for management decision making; however, their use is not widespread since they are expensive and laborious and they can only be performed on a few plants. VitiCanopy is a newly developed, free App for smartphones and tablet PCs that can be used as a simple alternative to traditional methods to measure canopy architecture parameters using digital image analysis. During the calibration and validation process, VitiCanopy has yielded highly comparable results to traditional methods and has been shown to reliably support the decision-making process for management purposes. Implementing VitiCanopy during routine vineyard operations could be useful to assess spatial differences in canopy architecture within a vineyard and can be related to other viticultural management strategies, such as irrigation and fertilization. Routine information gathering can be also achieved using VitiCanopy to create a record/history of practices and responses from grapevines in order to make more informed decisions to achieve specific wine styles and vineyard uniformity.

SYM-41-03
UNRAVELLING THE GENETICS OF SODIUM EXCLUSION IN NORTH AMERICAN VITIS SPECIES TO IMPROVE GRAPEVINE ROOTSTOCK BREEDING STRATEGIES
Dunlevy J.1, Henderson S.2, Blackmore D.1, Walker R.1, Edwards E.1, Gillham M.2 and Walker A.1
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Large genetic diversity exists within the world’s 48 species of Vitis, which have evolved over millennia to adapt to unique environmental niches. The use of interspecific hybrids as grapevine rootstocks can capture this beneficial genetic diversity, thereby increasing viticultural productivity, while maintaining the elite berry-related genetics of domesticated V. vinifera cultivars grafted to them. Initially exploited for their conferred resistance to phylloxera, grapevine rootstocks derived from wild North American Vitis species can also confer nematode resistance and increased tolerance to drought and salinity. Most rootstocks used in Australia were bred in either Europe or the USA and, as such, are not always ideally suited to Australian conditions. The aim of CSIRO’s rootstock breeding program is to utilize marker-assisted selection to combine key traits in new rootstock genotypes specifically suited to Australian vineyards. Soil salinity is an important issue to the Australian wine industry, as traditional wine grape cultivars can suffer from decreased growth and yield, and reduced berry quality due to high accumulation of damaging sodium and chloride ions when grown on saline soils. Fortunately, some rootstocks are able to limit the translocation and accumulation of these ions resulting in increased salt tolerance. Here we present the identification of a QTL and underlying candidate gene associated with the majority of variation in sodium exclusion in a complex interspecific rootstock family. The characterisation and genetic nature of four unique alleles, their species of origin, and implications for breeding work will be discussed.
SYM-41-04

USING HETEROLOGOUS EXPRESSION TO FUNCTIONALLY CHARACTERISE PROTEINS INVOLVED IN GRAPEVINE SALT EXCLUSION

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Genetic mapping, and comparison of whole root transcriptomes, has revealed a number of candidate genes that are likely to control sodium and chloride exclusion in grapevine rootstocks. These traits are important for grapevine salt tolerance in the field, and for producing superior wines that contain low salt concentrations. Here we present detailed functional data for proteins encoded by two candidate genes for salt exclusion (HKT and CCC) using heterologous expression systems. Expression of GFP-tagged proteins in tobacco (Nicotiana benthamiana) leaves, has been used to determine the subcellular localisation of the proteins. Furthermore, expression in yeast (Saccharomyces cerevisiae) and Xenopus laevis oocytes has been used to determine the substrate specificity for both proteins. Using two electrode voltage clamping, we analysed HKT allelic variants and site-directed mutants, and identified key amino acid residues that determine the sodium transport capacity of the protein. These findings correlate with the sodium-exclusion capacity of grapevine rootstock hybrids, where the presence of “faster” HKT variants in the plant genome enhances leaf sodium exclusion under saline irrigation. CCC, on the other hand, is unlikely to mediate chloride exclusion in Vitis species. These findings have the potential to aid molecular marker development for grapevine rootstock breeding programs.

SYM-41-05

MANIPULATING SOURCE-SINK RELATIONS WITH LOW CO2 SLOWS RIPENING OF SHIRAZ GRAPE BERRIES BUT DOES NOT UNCOUPLE ANTHOCYANIN PRODUCTION FROM SUGAR IMPORT

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With perennial fruit crops, many cultural practices are directed at yield regulation and optimising canopy structure. Photosynthetic output relative to yield largely determines the rate of sugar import to fruit, and may influence the production of secondary metabolites. Environmental factors such as sunlight exposure also influence fruit composition, but separating these effects is difficult under field conditions. In the wine industry, which devotes significant effort to managing yield and fruit exposure, understanding how sugar accumulation is linked to secondary metabolite production is important. If desirable metabolites can be increased in relation to sugar or maintained at higher yields, it would assist with increasing production efficiency and counteracting advancing ripening occurring under climate change. To specifically target carbohydrate supply effects on berry composition, an experimental system was devised to slow berry sugar accumulation without changing canopy structure or yield. This consisted of six transparent chambers to enclose large pot-grown grapevines and soda lime filled scrubbers that reduced CO2 concentration of day-time supply air by ca. 200 ppm compared to ambient supplied controls. When installed from veraison, berry sugar accumulation and anthocyanin production slowed proportionally to photosynthesis. There was no change in key regulatory gene expression, suggesting anthocyanin production was limited by metabolic precursor supply. Reducing CO2 therefore appears an effective experimental approach for studying carbohydrate supply effects on fruit composition.

SYM-42-01

PHOSPHATIDYLSERINE ‘SAVE-ME’ SIGNALS DRIVE FUNCTIONAL RECOVERY OF SEVERED AXONS IN CAENORHABDITIS ELEGANS

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Functional regeneration after axonal injury requires transected axons to regrow and re-establish connection with their original target tissue. The spontaneous regenerative mechanism known as axonal fusion provides a highly efficient means of achieving targeted reconnection, as a regrowing axon is able to recognize and fuse with its own detached axon segment, thereby rapidly re-establishing the original axonal tract. Here we use behavioral assays and fluorescent reporters to demonstrate that axonal fusion enables full recovery of function following axotomy of Caenorhabditis elegans mechanosensory neurons. Furthermore, we reveal that the phospholipid phosphatidyserine, which becomes exposed on the damaged axon to function as a ‘save-me’ signal, defines the rate of axonal fusion. We also show that successful axonal fusion correlates with the regrowth potential and branching of the proximal fragment, and with the retraction length and degeneration of the separated segment. Finally, we identify discrete axonal domains that vary in their propensity to regrow through fusion, and demonstrate that the rate of axonal fusion can be genetically modulated. Taken together, our results reveal that axonal fusion restores full function to injured neurons, is dependent on exposure of phosphatidyserine signals, and is achieved through the balance between regenerative potential and rate of degeneration.

SYM-42-02

REVEALING THE HOX CODE IN DEVELOPING SPINOCEREBELLAR NEURONS

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Coordinated body movement requires integration of many sensory inputs. This includes proprioception, the sense of relative body position and force associated with movement. Proprioceptive information of the lower body/hindlimb is relayed directly to the cerebellum via spinocerebellar (SC) neurons, located within four major neuronal columns or various scattered cell populations of the spinal cord. Despite their importance, a molecular understanding of these relay neurons is only beginning to be explored, with limited knowledge of molecular heterogeneity within and between columns. Here, we identify expression of Hox cluster genes, including both protein-coding genes and microRNAs, within SC neurons. Using neuronal tracing, in situ hybridisation and novel fluorescent reporter knock-in mice, we show that all posterior Hox genes of the 9-11 paralogs are expressed in SC neurons, revealing a “Hox code” based on axial level and individual SC column. Furthermore, we show that Hoxc9 function is required in most, but not all, cells of the major thoracic SC column, Clarke’s column, revealing heterogeneity reliant on Hox signatures.
NEUROGENIC DIFFERENTIATION BY HIPPOCAMPAL NEURAL STEM AND PROGENITOR CELLS IS BIASED BY NFIX EXPRESSION

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Our understanding of the transcriptional program underpinning adult hippocampal neurogenesis is incomplete. In mice, under normal physiological conditions, adult hippocampal neural stem cells (AH-NSCs) generate neurons and astrocytes, but not oligodendrocytes. The factors limiting oligodendrocyte production, however, remain unclear. Here, we reveal that the transcription factor NFIX plays a key role in this process. NFIX is expressed by AH-NSCs, and its expression is sharply upregulated in adult hippocampal neuroblasts. Conditional ablation of Nfix from AH-NSCs, coupled with lineage tracing, transcriptomic sequencing and behavioral studies collectively reveal that NFIX is cell autonomously required for neuroblast maturation and survival. Moreover, a small number of AH-NSCs also develop into oligodendrocytes following Nfix deletion. Remarkably, when Nfix is deleted specifically from intermediate progenitor cells and neuroblasts using a Dcx-creERT2 driver, these cells also display elevated signatures of oligodendrocyte gene expression. Together, these results demonstrate the central role played by NFIX in neuroblasts within the adult hippocampal stem cell neurogenic niche, with this transcription factor promoting the maturation and survival of these cells, while concurrently repressing oligodendrocyte gene expression patterns in these neuronally lineage-restricted cells.

THE P2X7 RECEPTOR REGULATION OF ADULT HIPPOCAMPAL NEURAL PROGENITOR CELLS

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Identifying the signalling mechanisms that regulate adult hippocampal neurogenesis is an essential step towards understanding how new neurons are generated and integrated into existing cytoarchitecture. Here we examine the roles of the P2X7 receptor, a purinergic calcium channel, in regulating both neural progenitor proliferation and phagocytosis of apoptotic immature neurons. Primary cultures of hippocampal neural progenitor cells were characterised using immunocytochemistry, and functional activity of P2X7 receptors was demonstrated using calcium influx and ethidium bromide uptake assays, both canonical functions of this receptor. Live cell confocal microscopy revealed hippocampal neural progenitors as capable of phagocytosing fluorescent latex beads, and flow cytometry in conjunction with specific inhibitors indicated P2X7 receptors as capable of facilitating this phagocytosis. Finally, P2X7 receptors were activated with bzATP and the thymidine analogue EdU was used to observe a significant dose-dependent relationship between concentration and proliferation. Evidence presented here demonstrates that P2X7 receptors can function as scavenger receptors in the absence of ATP allowing neural progenitors to phagocytose their apoptotic peers during neurogenesis as well as governing rates of proliferation, possibly by regulating calcium dependent transcription factor activation. Taken together, these data present a dual role for P2X7 receptors during adult neurogenesis. Given the crucial role neurogenesis plays in the hippocampus, dysregulation may lead to memory deficits and both neurological and psychological disorders. Our research is the first to demonstrate a single receptor with these dichotomous signalling roles.

A NOVEL ROLE OF 14-3-3ζ IN INTERNEURON DEVELOPMENT

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Dysfunction in the function and formation of GABAergic cortical interneurons has been implicated as a central pathogenic mechanism in schizophrenia, and other, neurodevelopmental disorders. 14-3-3ζ is part of a family of highly conserved intracellular proteins, that bind to the phosphoserine/threonine sites on target proteins and is highly expressed in the brain. Interestingly, several findings in recent years implicate 14-3-3ζ as a candidate risk factor for schizophrenia including: 1) 14-3-3ζ is downregulated in post-mortem schizophrenic brain samples at the mRNA level; 2) 14-3-3ζ is downregulated across multiple neuroproteomic studies on schizophrenia patient samples; 3) linkage studies have implicated 14-3-3ζ family proteins in numerous neurodevelopmental disorders, and 4) genetic mutations in the gene encoding 14-3-3ζ (YWHAZ) have been found in schizophrenia patients. Previous studies have shown that 14-3-3ζ KO mice exhibit anatomical and behavioural traits akin to those seen in schizophrenia and other neurodevelopmental disorders. Here we expand on previous findings by identifying a novel role for 14-3-3ζ in interneuron development. A key observation of this study was a subtype specific reduction in parvalbumin expressing interneurons throughout the cortex of 14-3-3ζ KO mice. Through a series of molecular, biochemical and morphological studies and analyses of unique mouse mutants i identified defects in the specification and formation of interneurons during early brain development. Furthermore, my data fits with the notion that 14-3-3ζ regulates the non-canonical Sth signalling pathway via Rac1 to control interneuron development. Taken together, this work provides novel insight into the role of 14-3-3ζ in controlling interneuron development and hence identifies a novel role of 14-3-3ζ in the pathogenesis of schizophrenia.

UNDERSTANDING HOW CHANGES TO THE COMPOSITION OF CHROMATIN CONTROL ITS STRUCTURE

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Eukaryotic DNA is packaged into a condensed structure called chromatin. Chromatin is built from nucleosomes. A nucleosome is a~147 base pairs of DNA wrapped almost twice around an octamer of histone molecules; two copies each of histone H2A, H2B, H3, and H4. Nucleosomes are repeated to form arrays with flexible linker DNA in between nucleosomes. These arrays fold and compact into complex higher-order structures that ultimately form a chromosome. It is well-established that histones and DNA encode all the information necessary to generate regular compacted chromatin in vitro. Yet, it is now clear that in vivo chromatin is less uniform and regular than first expected due to myriad variations in the biochemical make-up of nucleosomes and additional factors that bind to and/or modify chromatin. One of the most intensively studied chromatin binding factors is heterochromatin protein 1 (HP1α). HP1α is typically associated with silenced heterochromatin regions of the genome and binds to histone H3 methylated at lysine 9 (H3K9me). H3K9me is necessary for HP1α recruitment to heterochromatin, but it is not sufficient, suggesting additional factors are involved. One candidate emerging as a potential regulator of HP1α recruitment is the linker histone H1.4. We are currently using a series of biochemical and biophysical techniques to dissect the mechanism of how H1.4 influences the association of HP1α with chromatin and how these interactions affect chromatin structure.
**SYMPOSIUM**

**THURSDAY**

**SYMPOSIUM**

**SYM-43-02**

**STRUCTURAL MECHANISM OF PARTIAL AGONISTS AND ANTAGONISTS OF PPARGAMMA FOR USE AS ANTIADIABETICS**

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Synthetic full agonists of PPARγ have been prescribed for the treatment of diabetes due to their ability to regulate glucose homeostasis and insulin sensitivity. While the use of full agonists of PPARγ has been hampered due to severe side effects, partial agonists and antagonists have shown promise due to their decreased incidence of such side effects in preclinical models. No kinetic information has been forthcoming in regard to the mechanism of full versus partial agonism of PPARγ to date and little structural and dynamic information is available which can shed light on the mechanistic difference between full and partial agonists as well as antagonists. We have used X-ray crystallography, cellular assays, hydrogen Deuterium Exchange (HDX), and Surface Plasmon Resonance (SPR) to probe the mechanism of several PPARγ partial agonists and antagonists. Our findings demonstrate that not only do partial agonists and antagonists act through distinct transcriptional mechanisms, they also demonstrate differences in structure, dynamics, and kinetics as compared to full agonists. 1. “X-ray Crystal Structure of Voglitazone bound to PPARα and PPAR Subtype Selectivity of TZDs.” Rajapaksha H, Bhatia H, Worden K, Petrovsky N, Bruning JB, Biochim Biophys Acta. (2017 May 9). 2. “Structure-Activity Relationship of 2,4-Dichloro-N-(3,5-dichloro-4-(quinolin-3-yloxy)phenyl)benzenesulfonamide (INT131) Analogues for PPARα Targeted Diabetics.” Frick RL, He Y, Rodriguez BB, Chang MR, Kuruvilla D, Ciesla A, Abell AD, Kamenecka TM, Griffin PR, Bruning JB. J Med Chem. (2017 May 22). 3. “PPAR Post-Translational Modifications Regulate Bone Formation and Bone Resorption.” Stechschulte LA, Czernik PJ, Rotter ZC, Tausif FN, Corzo CA, Marciano DP, Asieaian A, Zheng J, Bruning JB, Kame necka TM, Rosen CJ, Griffin PR, Lecka-Czernik B. EBioMedicine. (2016 Aug;10:174-84). 4. “SR2067 Reveals a Unique Kinetic and Structural Signature for PPARγ Partial Agonism.” van Marrewijk LM, Polvak SW, Hijnen M, Kuruvilla D, Chang MR, Shin Y, Kupchka TM, Griffin PR, Bruning JB. ACS Chem Biol. (2016 Jan 15);11(1):273-83. 5. “Structure mechanism for signal transduction in RXR nuclear receptor heterodimers at least six proteins with two or more paralogs of each protein routinely identified when the complex is purified from cell extracts. To understand the structure and function of NuRD, a map of direct subunit interactions is needed. Dozens of published studies have attempted to define direct inter-subunit connectivities. We propose that conclusions reported in many such studies are in fact ambiguous for one or several reasons. First, the expression of many NuRD subunits in bacteria is unlikely to lead to folded, active protein. Second, interaction studies carried out in cells that contain endogenous NuRD complex can lead to false positives through bridging of target proteins by endogenous components. Combining existing information on NuRD structure with a protocol designed to minimize false positives, we propose a conservative and robust interaction map for the NuRD complex. We also suggest a 3D model of the complex that brings together the existing data on the complex. The highlighted issues and strategies are also applicable to the analysis of a wide range of multi-subunit complexes.

**SYMPOSIUM**

**SYM-43-03**

**MECHANISM FOR RNA-MEDIATED REGULATION OF THE HISTONE METHYLTRANSFERASE POLYCOMB REPRESSIVE COMPLEX 2 (PRC2)**

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Interactions between epigenetic modifiers and long noncoding RNAs or mRNAs has been widely reported. Yet, functional studies to elucidate how, or if, such epigenetic modifiers are regulated by their RNA binding partners are often complicated by promiscuous RNA binding. The polycomb repressive complex 2 (PRC2) is a histone methyltransferase that trimethylates K27 of histone H3 to produce the H3K27me3 mark: an epigenetic mark that is essential for the maintenance of the repressed chromatin state of thousands of genes. PRC2 binds thousands of coding and noncoding transcripts, which were proposed to either recruit it to target genes for repression, or to evict PRC2 upon gene expression. We combined classical biochemical and biophysical approaches in vitro with analyses of epigenomics data for protein–RNA (FRIP-seq) and protein–DNA (ChIP-seq) interactions in vivo to identify how PRC2 recognises its target transcripts. This has led us to discover that multiple short tracts of consecutive guanines largely increase the affinity of PRC2 to RNA in vitro and significantly associate with PRC2 binding sites on RNA transcripts in vivo. PRC2 binding transcripts are enriched at PRC2-binding sites on chromatin and H3K27me3-modified nucleosomes, provides a means for RNA-mediated regulation of PRC2.

**SYMPOSIUM**

**SYM-43-04**

**REFINEMENT OF THE SUBUNIT INTERACTION NETWORK WITHIN THE NUCLEOSOME REMODELLING AND DEACETYLASE (NuRD) COMPLEX**

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The nucleosome remodelling and deacetylase (NuRD) complex is essential for the development of complex animals. NuRD has roles in regulating gene expression and repairing damaged DNA. The complex comprises at least six proteins with two or more paralogs of each protein routinely identified when the complex is purified from cell extracts. To understand the structure and function of NuRD, a map of direct subunit interactions is needed. Dozens of published studies have attempted to define direct inter-subunit connectivities. We propose that conclusions reported in many such studies are in fact ambiguous for one or several reasons. First, the expression of many NuRD subunits in bacteria is unlikely to lead to folded, active protein. Second, interaction studies carried out in cells that contain endogenous NuRD complex can lead to false positives through bridging of target proteins by endogenous components. Combining existing information on NuRD structure with a protocol designed to minimize false positives, we propose a conservative and robust interaction map for the NuRD complex. We also suggest a 3D model of the complex that brings together the existing data on the complex. The highlighted issues and strategies are also applicable to the analysis of a wide range of multi-subunit complexes.

**SYMPOSIUM**

**SYM-43-05**

**REGULATING TRANSCRIPTIONAL ACTIVITY BY PHOSPHORYLATION OF THE INTELLECTUAL DISABILITY AND SEIZURE ASSOCIATED ARX HOMOEODOMAIN TRANSCRIPTION FACTOR**

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The paired-type Aristotle-related homoeodomain (ARX) transcription factor has critical roles in development. Mutations in ARX give rise to intellectual disability, epilepsy and brain malformations. Here we demonstrate that ARX protein is phosphorylated and using mass spectrometry and in vitro kinase assays we identify serine at position 37, 67 and 174 as sites of modification. We demonstrate that phosphorylation is required for correct transcriptional activity of ARX. Using a transcriptome wide approach we analysed gene expression using phosphoablative mutants (alanines replacing serines) compared to ARX wild-type (ARX-WT) overexpressed in pancreatic alpha-TC cells. ARX-WT overexpression compared to non-transfected cells had 70 genes with significantly altered expression (Log2FC >+/-1.0, P-value <0.05). This highlights the loss of significantly regulated gene expression compared to non-transfected cells with overexpression of the double phosphoablative mutant Ser37Ala+Ser67Ala (26%) and Ser174Ala (39%), respectively. Compared to ARX-WT the Ser174Ala mutant significantly altered expression of an additional 45 genes, including transporters (Slc2a4), kinases and signalling molecules. Using yeast-2- hybrid (confirmed by CoIP) we identified PICK1 (Protein interacting with C kinase 1) as a novel interacting protein, binding C-terminal region of ARX. PICK1 is a scaffold protein that facilitates protein interaction of proline-rich motifs and the protein kinase C alpha (PKC- alpha). We confirm that ARX is phosphorylated by PKC-alpha and demonstrate this kinase phosphorylates ARX at serine 174. In conclusion, we show that ARX is phosphorylated at several sites, and that this modification is important for aspects of transcriptional activity. Phosphorylation at serine 174 occurs via PKC-alpha suggesting the binding of the specific protein partner PICK1 with the C-terminal region of ARX is required. We contend that how these modifications and lead to early-onset seizure phenotypes in affected children may be due to deficient phosphorylation and subsequent alterations in transcriptional capacity.
SYM-44-01
ADVANCES IN HUMAN EPIGENOMICS
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For the last twenty-five years an increasing amount of evidence has shown the relevance of epigenetics in cell biology and tissue physiology, being DNA methylation aberrations in cancer the flag-ship for the recognition of its disturbance in human diseases. From the candidate gene approaches, new powerful technologies such as comprehensive DNA methylation microarrays and whole genome bisulfite sequencing has recently emerged that have reinforced the notion of epigenetic disruption in the crossroad of many sickness. From the posterior-boy cases of MGMT and GSTP1 hypermethylation in the prediction of alkylating drug response and prostate cancer detection, respectively, to the personalized treatment of leukemia with small molecules targeted to fusion proteins involving histone modifiers, the field has walked a long path. The current talk will focus in the epigenetic profiling, basically at the level of DNA methylation and histone modifications, that is starting to provide clinical value in the diagnosis, prognosis and prediction of response to drug therapies. For cancer, we have already a wide view of the understanding DNA methylation events that expand beyond classical promoter CpG islands of tumor suppressor genes and we have a growing list of mutated chromatin remodeler genes that contributes to the tumorigenesis process. It is time to apply this knowledge in practical clinical situations like the diagnosis of cancers of unknown primary, the screening of malignancies in high-risk populations or a biomarker selection of the patients that should receive treatment with anticancer drugs. Beyond our comfort zone, we should be aware that chemical modifications not only affect the DNA molecule, but also RNA. The epigenetics of RNA or the analysis of the epitranscriptome represents a another recent step to understand the complex relationship between genotypes and phenotypes in human tumors.

SYM-44-02
FREQUENT lack of REPRESSION CAPACITY OF PROMOTER DNA METHYLATION IDENTIFIED THROUGH GENOME-WIDE EPIGENOMIC MANIPULATION
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It is widely assumed that the addition of DNA methylation at CpG rich gene promoters silences gene transcription. However, this conclusion is largely drawn from the observation that promoter DNA methylation inversely correlates with gene expression. The effect of forced DNA methylation on endogenous promoters has yet to be comprehensively assessed. Here, we induced the methylation of thousands of promoters in the genome of human cells using an artificial zinc finger-DNMT3A fusion protein, enabling assessment of the effect of forced DNA methylation upon transcription and histone modifications, and the durability of DNA methylation after the removal of the fusion protein. We find that DNA methylation is frequently insufficient to transcriptionally repress promoters. Furthermore, DNA methylation deposited at promoter regions associated with H3K4me3 is rapidly erased after removal of the zinc finger-DNMT3A fusion protein. Finally, we demonstrate that induced DNA methylation can exist simultaneously with euchromatin modifications and silenced only by further DNA methylation.

SYM-44-03
MODULATION OF EPITHELIAL PLASTICITY BY TARGETED EPIGENETIC EDITING IN BREAST CANCER
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Over the last decade the sequencing of thousands of breast cancer patients has led to the pre-emptive identification of regulatory regions, non-coding RNAs, and loci conferring risk of relapse. The integration of these purely descriptive ‘omic’ features of cancer has now opened the door to functionalised, precision-medicine approaches to gain mechanistic understanding as to how cancer cells remodel during relapse, and to ultimately reverse treatment resistance by means of durable, locus-specific, epigenetic reprogramming of cancerous cells towards a normal-like (therapy-sensitive) state. Here we will describe the generation of epigenetic editing tools to interrogate the relationships between epigenetic modifications and gene expression, and to functionally map regulatory regions such as promoters and enhancers, for both coding genes and small non-coding RNAs. Importantly, the reversible characteristics of epigenetic modifications offer an attractive therapeutic opportunity to reprogram complex phenotypes, such as metastatic behaviour, via targeted epigenome engineering. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) proteins adapted for epigenetic editing provides an unprecedented tool to regulate multiple genes and reprogram cell phenotypes. In this system catalytically defective dCas9 is fused to epigenetic modifying domains to target specific epigenetic marks to specific sites in the genome. Epigenetic editing of multiple tumour suppressor genes and oncogenes will be discussed, particularly for the reprogramming of complex yet plastic and reversible gene programs such as epithelial-to-mesenchymal transition (EMT) in breast cancer. Finally, we will describe the development of novel tumour-specific delivery systems for Cas9-dCas9 in mouse or model systems. These potential applications for the future treatment of metastatic breast cancers for which no cure is available.

SYM-44-04
ELUCIDATING THE REGULATORY ROLE OF MI1916 IN TRUNK-TO-TAIL TRANSITION
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Hox genes are master regulators of patterning along the anterior-to-posterior (A-P, head-to-tail) axis during development. Both the genes themselves, and their function in body plan control, are conserved across bilateria. While the Hox genes are the ultimate effector molecules, it is the signals and molecules controlling strict temporal activation of Hox gene expression that orchestrate how the main body axis is laid down. Revealing the identity of these signals, and the cis-regulatory elements through which they act, are fundamental to our understanding of developmental and evolutionary mechanisms. We have recently identified a novel mechanism that controls the timing of a major Hox code transition, the trunk-to-tail transition. The miR-196 family of microRNAs are embedded in Hox clusters and target Hox genes of the trunk region. Genetic deletion of all three miR-196 genes in mouse results in a predicted upregulation of this trunk Hox code, with a concomitant, yet unexplained delay in the tail Hox code. We now employ both in vitro and in vivo systems to elucidate how miR-196, and other key signals including Gdf11, control the genomic regulatory architecture of the trunk-to-tail transition. Our data indicates that miR-196 and Gdf11 signalling have additive effects in the precise timing of posterior Hox gene activation, resulting in constraint of vertebral number and thus defining body length.
**SYM-45-01**

**HDAC3 IS A CRITICAL REGULATOR OF LIPID OXIDATION IN THE SMALL INTESTINAL EPITHELIUM**

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Histone deacetylase 3 (HDAC3) regulates expression of lipid metabolism genes in multiple tissues, however its role in regulating lipid metabolism in the intestine is unknown. Intestine-specific HDAC3 knockout mice (HDAC3KO) have significantly reduced weight gain. Intestinal epithelial cells (IECs) from these mice display co-ordinate induction of genes involved in peroxisomal and mitochondrial β-oxidation, and have markedly reduced levels of multiple lipids, particularly triglycerides. HDAC3 deletion increases the rate of lipid oxidation in IECs ex vivo, and decreases lipid accumulation in IECs in mice fed a high fat diet. Several genes induced following HDAC3 deletion are transcriptional targets of PPAR and their expression can be additively induced by treating IECs with a PPAR agonist and HDAC3 inhibitor. These findings suggest that by de-repressing PPAR driven transcription and promoting lipid oxidation in IECs, HDAC3 deletion reduces weight gain by limiting systemic lipid uptake. Intestinal HDAC3 therefore represents a novel therapeutic target in obesity.

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**SYM-45-02**

**HETEROGENEITY OF THE LIPIDOME IN CLINICAL PROSTATE TUMOURS, AND ITS MODULATION BY ANDROGENS, REVEALED BY MALDI-MASS SPECTROMETRY IMAGING**

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Lipid metabolism is exquisitely sensitive to androgen signalling in prostate cancer cells and cellular lipid composition is significantly altered during prostate cancer development. Here we studied the lipidome of human prostate tumours, to determine whether alterations in cellular phospholipids are directly androgen regulated and are associated with clinical features of the tumours. Using ex vivo culture of human prostate tumours, we generated a cohort of matched explants from >90 patients, cultured in the presence or absence of the current androgen receptor antagonist enzalutamide. Lipids extracted from whole tumour homogenates were analysed for all major phospholipid species by electrospray ionisation tandem mass spectrometry. Analysis of a large cohort revealed characteristic enzalutamide-induced alterations in fatty acid chain elongation and saturation, and identified individual lipid species whose change in abundance was associated with an anti-proliferative response to the agent. As there was significant inter-patient heterogeneity observed in the lipid profiles and response to enzalutamide, we used Mass Spectrometry Assisted Laser Desorption/Ionisation (MALDI) imaging coupled with tissue histology to view the spatial distribution of distinct phospholipid species within discrete pathological regions of these tissues, enhancing the identification of lipid and metabolite alterations. Moreover, we directly imaged the distribution of enzalutamide throughout the tissue and showed that, when dissolved in the tissue culture media, the drug completely penetrates the tissue core within 2 hrs of culture. In summary, MALDI mass spectrometry imaging of human prostate tumour explants has not only provided spatial information on drug distribution previously unobtainable, it has yielded cell type-specific profiles of lipids and their altered abundance in response to androgenic targeting.
**SYM-45-04**

**ENDOSOMAL SORTING OF THE MEMBRANE CARGO β-SECRETASE TO THE RECYCLING ENDOSES IS REGULATED BY GGA1-DEPENDENT RECOGNITION OF A PHOSPHORYLATED DISLL MOTIF**

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The intracellular trafficking of β-secretase (BACE1) regulates the proteolytic processing of amyloid precursor protein (APP) and the generation of pathogenic amyloid-beta (Aβ) peptides. Internalised BACE1 is segregated from APP in early endosomes and the diversion of the membrane-bound BACE1 from the endo-lysosomal pathway to recycling endosomes represents an important transport step in the regulation of amyloid beta (Aβ) production. However, the mechanisms that regulate endosome sorting of BACE1 are poorly understood. Here we assessed the transport of BACE1 from early to recycling endosomes and have identified essential roles for the SNX4-mediated, signal independent pathway and for a novel signal-mediated pathway. The signal-mediated pathway is regulated by the phosphorylation of the acidic cluster-dileucine DISLL cytoplasmic tail motif of BACE1. The phosphomimetic S498D BACE1 mutant was trafficked to recycling endosomes at a faster rate compared with wild-type BACE1 or the non-phosphorylatable S498A mutant. The rapid transit of BACE1 S498D from early endosomes was coupled with reduced levels of amyloid precursor protein processing and Aβ production, compared with the S498A mutant. We show that the adaptor, GGA1, and retromer are essential to mediate rapid trafficking of phosphorylated BACE1 to recycling endosomes. In addition, the BACE1 DISLL motif is phosphorylated, and regulates endosomal trafficking, in primary neurons. Our findings demonstrate that post-translational phosphorylation of DISLL enhances the exit of BACE1 from early endosomes, a process mediated by GGA1 and retromer, and is important in regulating Aβ production.

**SYM-45-05**

**LINEAGE SPECIFIC FUNCTIONS OF A MICROCEPHALY GENE (WDR62) AND INTERACTIONS WITH AURORA A KINASE PROMOTE NEURAL STEM CELL PROLIFERATION AND BRAIN GROWTH**

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Studies on the inherited neural-specific disorder of autosomal recessive primary microcephaly (MCPH) have revealed genes with critical, non-redundant functions in embryonic brain growth. However, MCPH proteins are expressed widely in the brain and cell lineage-specific contributions to neurogenesis have not been previously investigated. In recent studies, we used Drosophila models to dissect the neural and gliogenic-specific functions of the second most commonly mutated MCPH gene, wdr62-repeat region 62 (wdr62). Interestingly, while the neural stem cell-specific depletion of Drosophila WDR62 homolog, reduced neural stem cell numbers, brain size was not altered. In contrast, glial lineage-specific depletion of WDR62 resulted in decrease numbers in both glial and neural-stem cell populations and a 40% reduction in brain volume. In gain-of-function studies, the ectopic WDR62 overexpression in glial cells increased neural stem cell numbers and brain size. We further demonstrated that cell type-specific WDR62 interactions with the master mitotic kinase, Aurora A kinase (AURKA), determined brain growth. The depletion of AURKA in neural stem cells drives brain overgrowth, which was suppressed by WDR62 co-depletion. In contrast, the co-depletion of WDR62 and AURKA in glial cells further reduced neural stem cell numbers and brain size compared to WDR62 depletion alone. This indicates that glial-specific WDR62 and AURKA co-operate to non-autonomously regulate neural stem cell populations for optimal brain growth. Thus, dissecting lineage-specific contributions of MCPH factors will be critical in unraveling the molecular and cellular basis of complex diseases such as microcephaly.

**SYM-46-01**

**PTPS AND STATS IN OBESITY AND CANCER**

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Primary liver cancer is the fifth most common cancer worldwide. Hepatocellular carcinoma (HCC) accounts for 90% of primary liver cancers and is refractory to nearly all available therapies with a 5 year survival rate of < 9%. Over the last 20 years, the incidence of HCC in economically developed countries has been increasing; HCC has nearly doubled in the US. The obesity epidemic accounts for as much as 50% of the increase in HCC in developed nations. The impact of obesity on cancers and is refractory to nearly all available therapies with a 5 year survival rate of < 9%. Over the last 20 years, the incidence of HCC in economically developed countries has been increasing; HCC has nearly doubled in the US. The obesity epidemic accounts for as much as 50% of the increase in HCC in developed nations. The impact of obesity and type 2 diabetes on HCC is thought to be due to the increased risk of non-alcoholic fatty liver disease (NAFLD) and the progression to non-alcoholic steatohepatitis (NASH), the more aggressive form of fatty liver disease characterised by chronic inflammation and fibrosis. The causes for the progression to NASH and HCC development in obesity remain unclear. Inflammation and oxidative stress occur in the liver in obesity/type 2 diabetes and reactive oxygen species (ROS) such as H2O2 can oxidise and inactivate protein tyrosine phosphatases (PTPs) for the promotion of tyrosine phosphorylation-dependent signalling. We reported previously that obesity-associated oxidative stress drives the activation and inactivation of PTPs such as TnTPT and PTP1B in the liver to promote STAT-1/3 signalling [1]. I will present data for ROS inactivating PTPs to promote STAT-1/3 signalling and the development of NASH and HCC in obesity. The results presented will define hepatic PTP oxidation/inactivation as a key mechanism for obesity-mediated NASH and HCC. References: 1 Gurzov, E.N., et al. (2014) Cell Metab 20, 85-102.

**SYM-46-02**

**INTERPLAY BETWEEN LSD1 ERASER ENZYME AND THE CHROMATIN TETHERED PKC THETA MEDIATED SIGNALING PLATEFORM IS CRITICAL FOR CANCER STEM CELL BIOLOGY**

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Complex regulatory networks control epithelial-to-mesenchymal transition (EMT) but the underlying epigenetic control is poorly understood. Lysine-specific demethylase 1 (LSD1) is a key histone demethylase that alters the epigenetic landscape. Here we explored the role of LSD1 in global epigenetic regulation of EMT, cancer stem cells (CSCs), the tumour microenvironment, and therapeutic resistance in breast cancer. LSD1 coupled to the nuclear PKC theta induced pangenic gene expression in networks implicated in EMT and selectively elicits gene expression programs in CSCs whilst repressing non-CSC programs. In vivo, chemotherapy reduced tumour volume, and when combined with an LSD1 inhibitor, abrogated the mesenchymal signature and promoted an adaptive and innate tumour immunoresponse. Circulating tumour cells (CTCs) from metastatic breast cancer (MBC) patients were enriched with LSD1 and pharmacological blockade of LSD1 suppressed the mesenchymal and stem-like signature in these patient-derived CTCs. Overall, LSD1 inhibition may serve as a promising epigenetic adjuvant therapy to subvert its pleiotropic roles in breast cancer progression and treatment resistance. Finally, I will present our recent novel novel single cell epigenetic tools for FFPE analysis from patient derived tissue biopsies as well as potential implications for the utility of epi-therapy in combination with immunotherapy and chemotherapy.
SYM-46-03

SOMATIC HYPERMUTATION OF THE YAP ONCOCENE IN CUTANEOUS MELANOMA

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Melanoma is usually driven by mutations in BRAF or NRAS that trigger hyperactivation of mitogen-activated protein kinase (MAPK) signalling. However, MAPK targeted therapies are not sustainably effective in most patients. Accordingly, characterizing mechanisms that co-operatively drive melanoma progression is key to improving patient outcomes. The Hippo signalling pathway regulates cancer progression via its central oncoproteins, YAP and TAZ. Although Hippo deregulation is common in human cancer, mutations in Hippo genes are rare. As YAP hyperactivation occurs in uveal melanoma, we investigated this oncogene in cutaneous melanoma. YAP protein expression was elevated in most benign nevi and primary cutaneous melanomas but present at only very low levels in normal melanocytes. In patient-derived xenograft and cutaneous melanoma cell lines, we observed variable reliance of cell viability on Hippo pathway signalling that was independent of classical melanoma driver mutations such as BRAF and NRAS. Finally, in genotyping studies, we observed significant variable reliance of cell viability on Hippo pathway signalling. However, MAPK targeted therapies are not sustainably effective in most patients. Accordingly, characterizing mechanisms that co-operatively drive melanoma progression is key to improving patient outcomes. The Hippo signalling pathway regulates cancer progression via its central oncoproteins, YAP and TAZ. Although Hippo deregulation is common in human cancer, mutations in Hippo genes are rare. As YAP hyperactivation occurs in uveal melanoma, we investigated this oncogene in cutaneous melanoma. YAP protein expression was elevated in most benign nevi and primary cutaneous melanomas but present at only very low levels in normal melanocytes. In patient-derived xenograft and cutaneous melanoma cell lines, we observed variable reliance of cell viability on Hippo pathway signalling that was independent of classical melanoma driver mutations such as BRAF and NRAS. Finally, in genotyping studies, we observed significant variable reliance of cell viability on Hippo pathway signalling that was independent of classical melanoma driver mutations such as BRAF and NRAS. Finally, in genotyping studies, we observed significant variable reliance of cell viability on Hippo pathway signalling.

SYM-46-04

P53 AND RB REGULATE CILIogenesis AND HEDGEHOG PATHWAY SENSITISATION IN DEVELOPMENT AND CANCER

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Aberrant activation of the Hedgehog (Hh) pathway is implicated in the initiation and progression of various cancers. Although mutations in Hh pathway components are described in some cancers, the majority of Hh-tumours exhibit Hh pathway activation in the absence of such mutations. In this context, constitutive pathway activation is driven by ligand-dependent signalling, where Hh ligands produced by cancer cells maintain pathway activation and self-renewal. Our data suggest that p53 and Rb, tumour suppressors commonly mutated in Hh-cancers, are strongly implicated in regulating ligand-dependent Hh signalling. We show that genetic inactivation of p53 and/or Rb in the developing mouse neural tube promotes ciliogenesis and expansion of the Nkx2.2 Hh-dependent ventral domain at E10.5, consistent with a Hh-gain of function phenotype. siRNA knockdown of p53 and/or Rb in mesenchymal stem cell line, C3H10T1/2, promotes an increase in alkaline phosphatase, a marker of Hh-dependent osteoblast differentiation, in response to Sonic hedgehog ligand (Shh). Furthermore, p53 and/or Rb deletion in mouse embryonic fibroblasts or mouse osteosarcoma cell lines leads to an increase in primary cilia and an enhancement of Gli1 mRNA expression in response to Shh. Inhibition of Hh signalling using the potent Smoothened (Smo) inhibitor, LDE-225, completely blocks this response. Lastly, genetic inactivation of Smo in the p53;Rb conditional genetic mouse osteosarcoma model leads to a non-malignant phenotype and prolonged survival. Together, our data implicates p53 and Rb as genetic biomarkers for Hh ligand-dependent sensitivity and potential responsiveness to Hh-inhibitor therapy.

SYM-46-05

CIB2 IS A NEGATIVE REGULATOR OF ONCOGENIC SIGNALLING BY SPHINGOSINE KINASE 1 IN OVARIAN CANCER

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Sphingosine kinase 1 (SK1) plays an important role in cancer as a key regulator of the cellular balance between pro-apoptotic and pro-survival sphingolipids. Oncogenic signalling by SK1 relies on its localisation to the plasma membrane, which is mediated by calcium and integrin binding protein 1 (CIB1) via its Ca2+-myristoyl switch function. Here we show that another member of the CIB family, CIB2, plays a surprisingly opposite role to CIB1 in the regulation of SK1 signalling. We found that CIB2 interacts another member of the CIB family, CIB2, plays a surprisingly opposite role to CIB1 in the regulation of SK1 signalling. We found that CIB2 interacts with CIB1 through the same binding site on SK1 as CIB1, but lacks the Ca2+-myristoyl switch function. As a result, CIB2 blocks translocation of SK1 to the plasma membrane and inhibits its subsequent signalling, which includes sensitisation of cells to TNFa-induced apoptosis and inhibition of Ras-induced neoplastic transformation. We found that CIB2 is significantly down-regulated in ovarian cancer, and that low CIB2 expression is associated with poor prognosis in ovarian cancer patients. Notably, re-expression of CIB2 in ovarian cancer cells blocks plasma membrane localisation of endogenous SK1, reduces in vitro neoplastic growth, tumour growth in mice, and suppresses cell motility and invasiveness in both in vitro and in vivo assays. Furthermore, consistent with the in vitro synergistic effects between the SK1 specific inhibitor, SK1-I, and standard chemotherapeutics, re-expression of CIB2 also sensitises ovarian cancer cells to carboplatin. Together, these findings not only identify CIB2 as a novel endogenous regulator of SK1 signalling and potential prognostic marker, but also further demonstrate the therapeutic potential of SK1 in this gynaecological malignancy.

SYM-47-01

BREEDING FOR CROP PRODUCTIVITY: TECHNOLOGY INTEGRATION FOR IMPROVEMENTS IN GENETIC GAIN

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Breeding for improvements in crop productivity requires a balance of multiple components, ensuring yield gains are matched with demands for end-product performance. To increase genetic gain there are four key drivers to consider: (i) the level of genetic variance within a breeding program; (ii) the intensity within which a program is able and/or willing to select from the available germplasm; (iii) the accuracy with which selections are made; and (iv) the time taken to complete a breeding cycle. Complementary to these drivers are next-generation technologies that are being developed within the Australian research and development cycle. Complementary to these drivers are next-generation technologies that are being developed within the Australian research and development cycle. Complementary to these drivers are next-generation technologies that are being developed within the Australian research and development cycle. Complementary to these drivers are next-generation technologies that are being developed within the Australian research and development cycle.
Warming trends involve a gradual increase of temperature and higher incidence of heat waves. Working at different scales, from continental trial networks to detailed physiological experiments we illustrate the value of (i) separating stressful vs. non-stressful temperatures, (ii) identifying sensitive stages for impact on yield and (iii) understanding the interaction with other weather/soil factors. Using National Variety Trials across Australia we observed a consistent negative association between maximum temperature and yields in wheat, barley and canola with clear regional patterns. Days exceeding 30°C were unlikely before flowering; while canola and chickpea sampled the highest occurrence during grain filling, chickpea was less affected. In addition, a rise in minimum temperature during the critical period can lead to substantial yield reduction linked to a shorter cycle. Our study highlights that temperature in the non-stressful range can be associated with yield reduction with crop specific effects. Finally, the interaction between temperature and water stress presented a regional pattern. Under a Mediterranean rainfall pattern, high minimum temperature before flowering was associated with higher yields in wheat, barley, canola and chickpea, possibly promoting early growth and water use and reducing direct evaporation from the soil. Where crops depend on initial soil moisture, high yields were associated with lower minimum temperature, possibly slowing growth and early water use, lessening terminal stress. We discuss likely selection targets for temperature responsiveness per se that could add value to adaptation strategies.
SYM-48-01

GENERATION OF NON-GM YEAST THAT IMPART “ROSE” AROMAS IN WINE

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It is well established that the choice of yeast used to perform wine fermentation impacts significantly on sensory attributes of wines; different yeast species and strains impart different profiles of esters, volatile fatty acids, higher alcohols and volatile sulfur compounds. Indeed, this remains one of the simplest means by which winemakers can modulate the sensory attributes of wine. As a consequence, there are more than 100 commercially available Saccharomyces cerevisiae wine yeast strains available, mostly derived by isolation from vineyards and successful fermentations. Nevertheless, some desirable characteristics are not present amongst existing strains. The higher alcohol 2-phenylethanol (2-PE) and its acetate ester, 2-phenylethyl acetate (2-P EA), are derived from the aromatic amino acid phenylalanine and confer desirable “rose”, “floral” and “honey” aromas in wine. Natural and chemically mutagenised populations of a popular S. cerevisiae wine strain, AWRI 796, were subsequently exposed to toxic analogues of phenylalanine. Resistant colonies were found to overproduce 2-PE and 2-P EA by up to 30-fold in pilot-scale winemaking trials. Sensory analysis of the wines indicated that enhancing “floral” aromas was generally favourable for white varieties. Genome sequencing of these newly developed strains alongside existing wine strains revealed mutations in some of the genes in the biosynthetic pathway of aromatic amino acids, and several others that appear to mediate natural variation across S. cerevisiae for this oenologically important characteristic.

SYM-48-02

ALL YEASTS ARE EQUAL, BUT SOME YEASTS ARE MORE EQUAL THAN OTHERS

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Scientific interest in non-Saccharomyces yeasts is on the rise due to their uncommon physiological and metabolic functions. One species with remarkable, yet underexplored, biotechnological potential is Lachancea thermotolerans (formerly Kluyveromyces thermotolerans). It is an ubiquitously isolated from a range of natural and anthropic habitats covering a large geographic span. To gain insight into L. thermotolerans population diversity and structure, 172 isolates sourced from diverse isolation substrates worldwide were analysed using a set of 14 microsatellite markers. The resultant clustering revealed that the evolution of L. thermotolerans has been shaped by the geographical localisation, anthropisation and flux between different ecosystems. Genetic proximity of isolates originating from anthropic environments in particular grapes and wine, is suggestive of domestication events within the species. The observed clustering was further validated by several means, including population structure analysis, F-statistics, Mantle’s test and the analysis of molecular variance (AMOVA). Further support for the genetic clustering was provided via plate-based assays testing growth on several substrates and physicochemical conditions, followed by an in-depth phenotypical characterisation of isolates in an oenological context.

SYM-48-03

YEAST AS A PLATFORM FOR TERPENOID PRODUCTION

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Terpenoids are the most diverse class of natural compounds and have many current and potential applications. They are universally built from 5-carbon (C) isoprene units, via the precursors isopentenyl pyrophosphate (IPP) and/or dimethylallyl pyrophosphate (DMAPP). Monoterpenes (C10) and sesquiterpenes (C15) are of particular interest due to their many uses. They are found in plant essential oils, and are commonly used in industry as commodity chemicals and in daily life as pharmaceuticals, flavourings and fragrances. As an alternative to extraction from plant sources or chemical synthesis, the budding yeast Saccharomyces cerevisiae was engineered for their bulk production. In this work, the sesquiterpene trans-nerolidol and the monoterpene limonene were produced as the target terpene products. Other plug-in combinatory engineering will further improve the yeast platform efficiency for profitable industrialization.

SYM-48-04

NEW INSIGHTS INTO THE PROCESS AND FUNCTION OF TRYPTOPHAN C-MANNOSYLETION

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Tryptophan C-mannosylation is an unusual metazoan co-translational modification found on thrombospondin repeats (TSRs), type-I cytokine receptors and various other proteins, including enzymes like RNase-II and hyaluronidases. Tryptophan C-mannosylation transfers, integral membrane proteins encoded by the dpy19 genes, localise to the ER and use dolichol-phosphate mannose (Dol-P-Man) to glycosylate the indole-C2 of the N-terminal tryptophan within the canonical WXWX motif. Beyond this, little is known about these enzymes or the function of this protein modification. To better understand C-mannosylation, we have engineered these glycosyltransferases into the yeast Pichia pastoris. The enzyme can commandeer endogenous Dol-P-Man to modify recombinant proteins with a WXWX motif expressed in these yeast. This has enabled us to produce tens-of-milligrams of homogenous recombinant glycoproteins either with or without Trp C-mannosylation. The homogenous glycoforms have provided us the means to examine how C-mannosylation impacts protein stability, function and enzymatic activity. Our results suggest that the modification’s chief role is to stabilise protein structure. Using microsomal fractions from these engineered yeast strains, we have also established an in vitro assay of C-mannosyltransferase activity. We have used this assay to probe enzyme substrate preference and reveal that they are more promiscuous enzymes than previously appreciated. We have also performed site directed mutagenesis to identify residues that are essential for enzyme activity and inform models of enzyme mechanism. Finally, the assay and enzyme substrate preference data have facilitated the development of the first C-mannosyltransferase inhibitor: a chemical tool of great use for studying C-mannosylation both in vitro and in vivo.
SYM-49-02

ZEBRAFISH REGULATORY T CELLS MEDIATE ORGAN-SPECIFIC REGENERATIVE PROGRAMS


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The attenuation of ancestral pro-regenerative pathways may explain why humans do not sufficiently regenerate damaged organs. Vertebrate lineages that exhibit robust regeneration, including the teleost zebrafish, provide novel insights into the maintenance of adult regenerative capacity. Using established models of spinal cord, fin, and heart regeneration, we discovered that zebrafish Treg-like (zTreg) cells rapidly homed to damaged organs. Conditional ablation of zTreg cells blocked organ regeneration by impairing precursor cell proliferation. In addition to modulating inflammation, infiltrating zTreg cells stimulated regeneration through Interleukin-10-independent secretion of organ-specific pro-regenerative factors. Recombinant regeneration factors rescued the regeneration defects associated with zTreg cell depletion, whereas zTreg cells lacking a transcription factor Foxp3a infiltrated damaged organs but failed to express regenerative factors. Our data delineate novel organ-specific roles for Treg cells in maintaining pro-regenerative capacity that could potentially be harnessed for diverse regenerative therapies.

SYM-49-03

DELIVERING MORPHOGENS AND IMMUNOMODULATORS TO PROMOTE TISSUE REGENERATION


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Current trends in regenerative medicine are focused on generating tissue specific stem cells to repair/regenerate damaged tissues and organs. However, given the complexity of most tissues and organs, the ideal tissue regenerative stem cell would be one that was sufficiently plastic to contribute to the repair of multiple tissue types in a context dependent manner. We have developed a vector and transcription factor free method using a demethylating agent (5-azacytidine (AZA)) and a cytokine (platelet derived growth factor (PDGF)-AB) to reprogram terminally differentiated somatic cells into multipotent stem (iMS) cells by synergistically activating the JAK/STAT and JNK/c-JUN pathways. Murine iMS cells contribute directly to in vivo tissue regeneration in a context dependent manner without scar formation or malignant transformation (Chandrakanthan et al. PNAS 2016). This method has now been modified to reprogram human primary adipocytes into iMS cells in xenofree conditions. These iMS cells display a stable karyotype and can be expanded in serum-free conditions, display colony forming unit potential, serial re-plating ability and multi-lineage differentiation. Unlike human iMS cells which have been shown to form tumours when implanted in the immunocompromised NOD-SCID mouse, these human iMS cells formed active, functional new blood vessels, bone, cartilage and smooth muscle at sites of injury.

SYM-49-04

GENERATING MULTIPOTENT STEM CELLS FROM PRIMARY HUMAN ADIPOCYTES FOR TISSUE REGENERATION


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Current trends in regenerative medicine are focused on generating tissue specific stem cells to repair/regenerate damaged tissues and organs. However, given the complexity of most tissues and organs, the ideal tissue regenerative stem cell would be one that was sufficiently plastic to contribute to the repair of multiple tissue types in a context dependent manner. We have developed a vector and transcription factor free method using a demethylating agent (5-azacytidine (AZA)) and a cytokine (platelet derived growth factor (PDGF)-AB) to reprogram terminally differentiated somatic cells into multipotent stem (iMS) cells by synergistically activating the JAK/STAT and JNK/c-JUN pathways. Murine iMS cells contribute directly to in vivo tissue regeneration in a context dependent manner without scar formation or malignant transformation (Chandrakanthan et al. PNAS 2016). This method has now been modified to reprogram human primary adipocytes into iMS cells in xenofree conditions. These iMS cells display a stable karyotype and can be expanded in serum-free conditions, display colony forming unit potential, serial re-plating ability and multi-lineage differentiation. When transplanted in the NOD-SCID mouse using a postero-lateral inter-lumbar vertebral injury model, iMS cells were retained at the transplant site for more than a year with no evidence of metastasis or spontaneous teratoma formation. Transplanted human iMS cells contribute to the formation of new blood vessels, bone, cartilage and smooth muscle at sites of injury.
SYM-49-04

FLIGHTLESS I REGULATION OF PERICYTE FUNCTION IN DIABETIC CHRONIC WOUNDS

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Wound management in Australia costs over $2.6 billion/annum, with costs escalating due to the increase of diabetes in our society. Flightless I (Flii) is a protein which impairs healing responses, however, its role in diabetic wound healing is still poorly understood. Pericytes regulate inflammation and angiogenesis, two key processes involving Fili which are commonly dysregulated in diabetic wounds. Here, we aimed to understand the effects of Fili on pericyte function in diabetic wounds. Wild-type and Fili heterozygous knockout mice, treated with streptozotocin to induce diabetes, were wounded and the effect on pericyte numbers and function assessed. Increased numbers of pericytes were observed when Fili was decreased. This coincided with decreased leukocyte infiltration and increased expression of pro-angiogenic markers. These results suggest that inhibiting Fili may increase pericytes within wounds, restoring angiogenic and inflammatory regulation lost through diabetes. Understanding wound repair mechanisms will hopefully lead to new treatments for diabetic wounds.

SYM-49-05

HSPGS AS DRIVERS OF NEURAL PROGENITORS

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Heparan sulfate proteoglycans (HSPGs) are a family of ubiquitous proteins mediating a number of vital cellular processes including proliferation and stem cell lineage differentiation. Human mesenchymal stem cells (hMSCs) cultured in basal conditions (undifferentiated monolayer cultures) co-express neural markers and HSPGs throughout expansion with the addition of exogenous HS influencing cellular HSPG and neural marker expression. Conversion of hMSCs into hMSC Induced Neurospheres (hMSC IN) suggest that in vitro generated hMSC IN may represent an intermediary neurogenic cell type with HSPGs associated with hMSC IN formation. We have also investigated the effect of PDGF-B on human neuron development, in comparison to the common neuronal supplement BDNF. With both BDNF and PDGF reported to bind HS, we also investigated the potential modulatory effects of co-stimulation with the HS-analogue heparin to further modulate the effects of BDNF or PDGF-B. Using long-term (D40 and D60) neuronal differentiating cultures of human embryonic stem cell (ESC)-derived NSCs, we examined generated neurons for differences in viability, proliferation, heterogeneity, lineage marker expression as well as spontaneous calcium (Ca2+) signalling activity. Our results identified key phenotypical differences induced by BDNF and PDGF-B with the results supporting the use of PDGF-B in the generation subtype specific neurons. The identification of factors regulating neural lineage specification may provide new strategies for their efficient implementation in therapeutic applications.

SYM-50-01

O-FUCOSYLATION OF PLASMODIUM FALCIPARUM VIRULENCE PROTEINS ENSURES EFFICIENT INFECTION OF MOSQUITO AND VERTEBRATE HOSTS

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Malaria causes over 650,000 deaths annually and is transmitted between humans by mosquitoes. The sporozoite form of the malaria parasite is deposited into the human skin and rapidly migrates to the liver and infects hepatocytes. Liver stage parasites egress after approximately one week and establish a malaria infection in red blood cells. O-glycosylation of the Plasmodiumsporozoite virulence proteins CSP and TRAP was recently demonstrated, but the role of this modification in the parasite life cycle and its relevance to vaccine design remain unclear. Here, we identify the Plasmodium protein O-fucosyltransferase (POFUT2) responsible for O-glycosylating CSP, TRAP and other Plasmodium virulence proteins. Genetic disruption of POFUT2 in Plasmodium falciparum results in parasites that are attenuated for colonising the mosquito midgut, an essential step in malaria transmission. Some POFUT2-deficient parasites can infect the mosquito and they mature into salivary gland sporozoites. However, the sporozoites are impaired for normal virulence traits required for liver infection, including gliding motility, cell traversal and hepatocyte invasion. Loss of O-glycosylation also significantly reduces sporozoite infectivity and fitness in humanized chimeric liver mice. These defects can be attributed to destabilization and incorrect trafficking of virulence proteins bearing thrombospondin repeats (TSRs), including CSP and TRAP. Therefore, POFUT2 plays a similar role in malaria parasites to that in metazoa; it ensures the trafficking of Plasmodium TSR virulence proteins as part of a non-canonical glycosylation-dependent ER protein quality control mechanism.

SYM-50-02

COMPLEX ADAPTIVE LIFESTYLES PSEUDOMONAS AERUGINOSA AFFECTING VIRULENCE AND ANTIBIOTIC SUSCEPTIBILITY

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Pseudomonas aeruginosa can adapt its lifestyle to many different circumstances using a wide variety of genes, a process we have termed complex adaptation; indeed its promiscuity as an opportunistic pathogen seems to reflect this. We are interested in 4 types of adaptations, biofilm formation on surfaces (responsible for two thirds of all infections), swarming motility and mucin-enhanced surfing motility on surfaces (presumably involved in establishment of infections) and adaptation to in-vivo-like growth conditions. Each of these adaptations causes altered expression of hundreds of genes, including those mediating virulence factors and antibiotic resistance determinants as well as metabolism, and are dependent on hundreds of genes as revealed by screening ordered mutant libraries. Intriguingly we have been able to identify several regulatory loci that control not only antibiotic resistance but also virulence and often central metabolism, including lon, cbrAB, phoPQ, ntrBC etc; several of these loci appear to perform as switches between chronic (biofilm) and virulent (swarming) states of the organism. To enable the investigation of adaptive lifestyles in vivo and enable understanding of the key virulence factors as well as effective therapies for high density infections, we have developed a new simple mouse abscess model that enables the study of both chronic and invasive infections by all of the most resistant organisms in our society. Based on our earlier observation that human host defence peptide LL-37 can inhibit biofilm formation and dissolve existing biofilms, we screened for and obtained small protease-resistant peptides with potent broad-spectrum anti-biofilm activities and are also effective vs. swarming and surfing cells. Our novel peptides (i) kill the major multidrug-resistant bacteria in biofilms (MBEC <1ug/ml), (ii) work synergistically with antibiotics, (iii) are effective in animal models, and (iv) block the stringent response that controls biofilm formation and virulence.
SYM-50-03

CHARACTERISING NOVEL ANTIGENS FOR GONOCOCCAL VACCINE DEVELOPMENT

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Neisseria gonorrhoeae, the causative agent gonorrhoea, is a major public health problem worldwide with an estimated global incidence of 106 million cases/year. If left untreated, infection can lead to severe sequelae that include pelvic inflammatory disease, infertility, neonatal complications, and an increased risk of HIV. It recognised by WHO and CDC as an urgent threat to global health due to the emergence of multi-drug resistant gonococcal strains. There is currently no vaccine, and no new antibiotics or new vaccine candidates in late-stage development. To facilitate gonococcal vaccine development, we performed mathematical modelling to predict the impact of different vaccine scenarios. We have also identified and characterized a series of potential vaccine candidates. Mathematical modelling of different vaccine scenarios indicates that a more modestly efficacious vaccine could have a substantial impact on gonorrhoea prevalence and sequelae. We have also characterised 2 highly conserved and immunogenic candidate vaccine antigens. In vitro assays, using wild type, knock-out and complemented strains, have shown that NGO1958 (gonococcal homologue of the histone binding antigen (NHBA) that is present in the serogroup B meningococcal vaccine Bexsero) is involved in serum resistance and adherence to cervical epithelial cells. Similar assays show that NGO2139 (a methionine uncorrected receptor in resistance to killing by human serum, monocytes and macrophages, as well as adherence and invasion of cervical epithelial cells. Antibodies to these proteins are bactericidal and can block gonococcal infection of cervical epithelial cells. We present two recombinant protein antigens that elicit both bactericidal and functional blocking antibodies, which are valid candidate antigens for possible inclusion in an urgently needed vaccine for the prevention of gonorrhoea.

SYM-50-05

STRUCTURAL STUDIES TO INVESTIGATE THE ‘SUBSTRATE VERIFICATION’ ACTIVITY OF CLASS III BIOTIN PROTEIN LIGASES

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The covalent attachment of co-enzyme biotin onto certain metabolic enzymes is a post-translational modification that occurs throughout the living world. BPL is the enzyme responsible for protein biotinylation. Crystal structures of class I and II BPLs, present in archaea and bacteria, have been reported. However, the class III BPLs, found in mammmals, fungi and insects, have not been as extensively structurally characterised. These BPLs contain a catalytic domain that is conserved between the 3 classes, but also have a large N-terminal extension that appears to assist with selection of appropriate targets for biotinylation. The paucity of structural information means the molecular basis for the N-terminal domain in substrate recognition is unknown.

Crystallography attempts have so far been unsuccessful. Therefore, we have applied alternative techniques to gain new insights into the structure and function of the model class III BPL from Saccharomyces cerevisiae (ScBPL). Homology modeling using Phyre suggests the BPL catalytic domain is structurally homologous to other BPLs from class I/II, whilst the N-terminal domain shares the fold of the glutamine amidotransferase subunit from pyridoxal-5'-phosphate synthase. The glutamine amidotransferase catalytic residues involved in the conversion of glutamine to glutamate and ammonia are conserved and correctly positioned in the ScBPL N-terminal domain model. However, preliminary 1H 1D NMR has demonstrated ScBPL does not contain the catalytic activity. Ligand observed NMR techniques and activity assays are being employed to determine if this catalytic site retains affinity for glutamine and other glutamine mimics. Furthermore, mass spectrometry (MS) surface labelling techniques are being used to validate the homology models, whilst mutagenesis, native and cross-linking MS studies, and NMR spectroscopy will be employed to generate a structural view of how the N-terminal domain of ScBPL interacts with substrate. Understanding this process will provide valuable insights into this precise post-translational modification.

SYM-50-04

ASPERRILUS FUMIGATUS THIOREDUCTASE: A POTENTIAL ANTIFUNGAL DRUG TARGET

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Aspergillus fumigatus infections are increasing in incidence and are associated with high mortality rates, high cost of treatment and longer hospital stays. In addition, the limitations of current effective antifungals and increasing antifungal resistance highlight the need for the development of new antifungals with novel targets. Thioreductase catalyses the reduction of thioredoxin by NADPH, and is responsible for maintaining a reducing intracellular environment and acting as an electron donor for various biosynthetic enzymes. It has been shown to be essential for the growth of A. fumigatus and therefore presents as a potential target for novel antifungals. The aims of this project are to gain a detailed knowledge of the molecular mechanisms underlying its function by X-ray crystallography, and identify small molecule inhibitors that may serve as lead compounds for the development of novel antifungals. To date, we have successfully solved the structure of A. fumigatus thioreductase in complex with FAD and NADPH to 3.2Å, and shown that Ebselen - a small drug-like molecule - is a nanomolar inhibitor of the enzyme in vitro and also inhibits growth of A. fumigatus with an MIC of 1-2 μg/mL. Using mass spectrometry we have also demonstrated that Ebselen interacts covalently with a specific catalytic cysteine. Current work is focussed on solving the structure of the enzyme in complex with Ebselen in order to define interactions at an atomic level which are important for inhibition of the enzyme, providing a scaffold for future design of specific and potent anti-Aspergillus drugs that target thioreductase.

SYM-50-01

CHROMATIN MODIFIERS SET-32 AND SET-25 ESTABLISH A TRANSGENERATIONAL SILENCING SIGNAL

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Mounting evidence in a number of organisms suggests that some epigenetic modifications acquired by an individual during its lifetime can be inherited for multiple future generations. This phenomenon is termed transgenerational epigenetic inheritance, and may provide a mechanism for the inheritance of environmentally acquired traits. We are studying transgenerational epigenetic inheritance using the nematode Caenorhabditis elegans. We have developed a system in which RNAi-induced silencing of a GFP transgene is robustly inherited for multiple generations in the absence of the initial RNAi trigger. We have shown that the histone methyltransferase SET-25 and the putative histone methyltransferase SET-32 are required for effective transmission of transgene silencing. Specifically, whilst set-25 and set-25 mutant animals exposed to RNAi display silencing of the GFP transgene, their unexposed offspring fail to inherit this silencing. Intriguingly however, the few animals which escape this failure and remain silenced then produce subsequent generations of silenced progeny. Furthermore, set-25 and set-25 mutants segregated from silenced set-25/+ and set-25/+ heterozygotes respectively remain fully silenced. Together, this data suggests that SET-25 and SET-32 are required for the establishment of a transgenerational silencing signal, but not for the long-term maintenance of this signal between subsequent generations. We thus propose a three-step model of transgenerational epigenetic inheritance consisting of Initiation, Establishment and Maintenance. In order to further support our model we have performed small RNA sequencing and proteomic studies on both SET-25 and SET-32 mutants and will also present these results.
SYM-51-02

EPIGENETIC DIVERGENCE CONTRIBUTES TO TIGER SNAKE ADAPTATION TO ISLAND ENVIRONMENTS

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While research on changes in phenotypic plasticity within wild animal populations is centuries old, very few studies have empirically demonstrated a role for epigenetic variation in the response of natural populations to environmental change. Here we identify a region of biogeographic complexity that houses unique genetic diversity in the wild tiger snake (Notechis scutatus) and examine how adaptation to island environments can occur through epigenetic divergence. These snakes have been isolated on a mosaic of offshore islands where they show a variety of phenotypic adaptations, including differences in skin colour, sexual dimorphism, scale counts, aggression levels and levels of plasticity in body and head size linked to the ability to ingest large prey. We have generated methylation sensitive amplified polymorphism (MSAP) data and found that, in general, tiger snakes on island groups that share an evolutionary history and environmental niche are more distinguished by methylation status than genetic differences. Tiger snakes on each island group have a distinctive epigenetic signal, suggesting local adaption to these environments. This is also supported by the strong positive relationship between differences in epigenetic profile and population isolation age, temperature in winter and precipitation level in summer.

SYM-51-03

EFFECT OF GEOGRAPHIC ORIGIN, PLANT AGE AND PROPAGATION SYSTEM ON THE DNA METHYLATION OF VITIS VINIFERA

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Epigenetic mechanisms are a key interface between the environment and plants genotypes. These mechanisms provide agility and plasticity by regulating gene expression in response to developmental and environmental changes, to ultimately affect the plant’s phenotype. When the epigenetic “memory” of the environment modifies the response to subsequent environmental cues, the plant is “epigenetically primed”. This is a recently discovered system by which plants can increase their resilience to challenge. It is now also widely accepted that epigenetic mechanisms have been the source of favourable traits during crop varietal selection. In grapevine, fruit traits associated to wine quality are largely determined by the fruit’s origin and, to a lesser extent, by the vine age. Although environmentally induced epigenetic variability has been shown to be partially maintained over meiotic generations in annual species, very little is known about the effect of different vegetative propagation systems on the maintenance of the epigenetic memory acquired with plant age in long-living species. Our results show that differences in methylation profiles between vineyards are driven by geographic distance between vineyards and differences in vine age. Our results also indicate that different propagation systems have different effects on the DNA methylation profiles of the new propagules and that such differences could be partially responsible for the observed differences in fruit quality between vineyards.

SYM-51-04

A SHORT PERIOD OF REPETITIVE MECHANICAL STRESS TRAINS A CELLULAR MEMORY TO PROMOTE THIGMOMORPHOGENESIS

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Prolonged mechanical stress (MS) activates molecular and physiological processes that alter plant morphology and development inducing thigmomorphogenesis. Previously, we reported that a histone and DNA architecture-modifying enzyme impaired thigmomorphogenesis and touch-responsive gene expression, raising the question of whether MS can program cellular memory formation? Here we demonstrate that 7 days of mechanical stimulation of juvenile seedlings was sufficient to reduce cell size and induce thigmomorphogenesis even in the absence of any further mechanosensation, A longer duration of MS enhanced touch-responsiveness gene expression, especially in juvenile seedlings undergoing cellular differentiation. Prolonged MS for 14 days affected the basal expression levels of touch responsive genes (AOS, CML39, GA20OX6, TCH3, TCH4), which persisted for 4 days following MS. These findings agree with the hypothesis that thigmomorphogenesis is an epigenetic phenomenon involving the memory of mechanosensation to newly differentiated naive cell types that facilitate stress accclimation.

SYM-51-05

PROGRAMMABLE DNA LOOPING USING ENGINEERED BIVALENT CAS9 COMPLEXES

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DNA looping is a ubiquitous and critical feature of gene regulation. Although DNA looping can now be efficiently detected, tools to readily manipulate DNA looping are lacking. Here, we report the development and use of a set of CRISPR-based DNA looping reagents for the creation of programmable DNA loops. Cleavage-defective Cas9 proteins of different specificity were linked by heterodimerization or translational fusion to create bivalent complexes able to link two separate regions of DNA. A statistical mechanical model was developed to describe bivalent DNA looping is a ubiquitous and critical feature of gene regulation. Although DNA looping can now be efficiently detected, tools to readily manipulate DNA looping are lacking. Here, we report the development and use of a set of CRISPR-based DNA looping reagents for the creation of programmable DNA loops. Cleavage-defective Cas9 proteins of different specificity were linked by heterodimerization or translational fusion to create bivalent complexes able to link two separate regions of DNA. A statistical mechanical model was developed to describe bivalent DNA looping. Overall looping efficiency could be significantly improved by loop multiplexing. As a proof-of-principle, the bivalent complexes were used to activate an inserted reporter gene by rewiring E. coli chromosomal DNA to bring a distal enhancer element, located ~12kb away, to the gene promoter. Such reagents should allow manipulation of DNA looping in a variety of cell types, aiding understanding of endogenous loops and enabling the creation of new regulatory connections.

PAGE 80
SYM-52-01
ROLE OF PROTEIN PHOSPHATASE PEZ IN REGULATING RECEPTOR TYROSINE KINASE TRAFFICKING

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Receptor tyrosine kinases (RTKs) are key regulators of fundamental cellular functions including proliferation and survival. Trafficking itineraries determine the amount of receptor on the cell surface available to transmit growth and survival signals, but signalling networks controlling these itineraries are not well understood. At steady-state, the amount of cell surface receptor reflects the balance between synthesis, endocytosis, recycling and degradation. Altered receptor trafficking underlies a number of pathologies and thus delineating mechanisms that regulate trafficking will highlight potential areas of intervention. Non-receptor protein tyrosine phosphatase Pez (PTPN14) is a developmentally-regulated protein that when reduced or lost gives rise to lymphoedema in mouse and humans. Mutations of Pez have been found in multiple cancers and we have demonstrated a role for Pez in suppression of metastasis by reducing intracellular protein trafficking through the secretory pathway. We have found that Pez localizes to subsets of endosomal compartments and has identified novel Pez regulated pathways that control EGFR expression on the surface of breast cancer cells. We have shown that loss of Pez is associated with stabilization of the EGFR protein, elevated P-ERK levels and enhanced anchorage independent growth of breast cancer cells. In addition, loss of Pez results in impaired endosome maturation and elevated EGFR recycling. Together this data suggests a sequestration of RTK away from the degradation pathway, in the absence of Pez, via a mechanism that changes the identity of the early endosomal compartment which is the site of the decision point between recycling and degradation. Since intracellular trafficking regulates the cell surface expression of multiple RTKs, understanding the molecular mechanisms that regulate trafficking routes could lead to therapeutic strategies that have the potential to target multiple RTKs simultaneously.

SYM-52-02
FLUORESCENT INTRA-BODY LOCALIZATION MICROSCOPY (FILM): A NOVEL METHOD FOR TRACKING SINGLE INTRACELLULAR ENDOGENOUS AND GFP-TAGGED PROTEINS IN VITRO AND IN VIVO

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By breaking the Abbe law of diffraction, super-resolution microscopy techniques provide unprecedented detail of biological structures and processes. However, the requirement for fluorescent photoconvertable tags has hampered progress and exposed PALM and STORM techniques to over-expression artifacts, raising the need for developing novel tools to bypass these limitations. Here, we describe the development of a single chain expressed in cells as intra-nanobodies to perform single molecule imaging of any GFP-tagged or endogenous intracellular proteins. Configuration 1: Co-expression of a GFP binding nanobody tagged with a photoconvertible mEOS2 to image any GFP-tagged protein in cells. We co-expressed anti GFP-intra-nanobody-mEos with PH-PLCδ-GFP allowed super-resolution imaging of phosphatidylinositol(4,5)bisphosphate nanodomains in fixed and live neurosecretory PC12 cells. We found identical domain size and mobility when using PH-PLCδ-mEos2. Further, combining the intra-nanobody with an Apex tag allowed us to perform 3D electron microscopy on these nanodomains. We also visualized cell-cell junctions at nanoscale and identified a modified C402 cell line expressing GFP tagged E-cadherin at endogenous levels. Expressing the GFP intra-nanobody, within the nematode C. elegans PLM mechanosensory neurons, allowed us to visualize the binding of cell-type specific proteins in vivo. In addition, using zebrafish D. rerio expressing the GFP intra-nanobody, we were able to visualize Caveolin 3 within its muscular fibril. Configuration 2: Purpose-designed intra-nanobodies to probe the nanoscale organization of endogenous proteins. Two specific nanobodies developed against the endogenous 3’-adenosine nucleotide, N60 and N37, were used to track endogenous receptors either in their active or inactive states. Fluorescent intra-body Localization Microscopy (FILM), therefore enables GFP-tagged constructs to be localized to in-vivo, super-resolution, avoiding clumping and aggregation problems for existing in vitro or in vivo models. This technique can also be extended to identify the diffusional signature and nanoscale organization of endogenous proteins by expressing selective purpose-designed intra-nanobodies.

SYM-52-03
THE MOLECULAR INTERPLAY BETWEEN CARGO RECOGNITION AND ACTIVITY OF THE MICROTUBULE MOTOR PROTEIN, KINESIN-1

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The correct spatial and temporal distribution of intracellular cargoes such as proteins, vesicles and organelles are essential for maintaining normal cellular function. Kinesin-1, the archetypal member of the kinesin superfamily of motor proteins, is a tetramer of two heavy chains (KHCs) and two light chains (KLCs) and is responsible for driving anterograde transport of cargoes along the microtubule network. Despite its critical role, the molecular mechanisms underlying regulation of kinesin-1 activity are not well understood, other than cargo interaction is sufficient to induce kinesin-1 motility. In the absence of cargo binding, kinesin-1 exists in a folded, auto-inhibited state achieved via an intramolecular interaction within the KHC component. Here, we identified a conserved Leu-Phe-Pro motif residing within an unstructured region of KLC that stabilizes this kinesin-1 motor. Using a fluorescence resonance energy transfer biosensor in combination with biochemical, biophysical, and X-ray crystallographic approaches, we describe an intramolecular interaction between this motif and the tetrapetide repeat (TPR) domain of KLC. This interaction also partly occludes a key cargo binding region on the TPR domain. Thus, upon cargo binding, this intramolecular interaction is displaced and results in a global conformational change within the KLCs. Thus, like KHCs, KLCs exist in a dynamic conformational state that is regulated by self-association and cargo binding. Taken together, our studies highlight how this molecular switch, cargo binding regulates the activity of the holoenzyme.

SYM-52-04
DESMOGLEIN-2 AND ITS ROLE IN TYPE 1 DIABETES: REVEALING A CRITICAL CROSS-TALK BETWEEN THE VASCULATURE AND INSULIN-PRODUCING BETA ISLET CELLS

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Pancreatic islet transplantation is a promising cellular therapy for type 1 diabetes; however, outcomes are limited by the rapid death of the insulin-producing beta cells and the poor revascularization of the islet graft once transplanted. Co-culture of endothelial cells (the cells that form the inner lining of blood vessels) with the insulin producing beta islet cells have been shown to improve beta cell survival and function in vivo. In light of this novel interaction, we investigated the role of the desmoglein-2 (DSG2), an important cell adhesion cadherin that mediates this islet-cadherin interaction between beta cells and the endothelial cells of the vasculature. Data suggest that DSG2 is expressed by both pancreatic beta islet cells and endothelial cells of the pancreas which implicates this adhesion molecules outside of its canonical role in desmosome formation. Our live-animal imaging data reveal that DSG2 is particularly important for blood vessel barrier integrity with the DSG2-loss-of-function strain of mice (DSG2+/-) exhibiting increased vascular permeability with increased leakiness of the 70kDa FITC-Dextran. Further investigation of these mice has revealed that the pancreatic endothelial cells are altered in morphology, and that in DSG2-/- mice have more susceptible to streptozotocin-induced diabetes. More recent work suggests that DSG2 is playing a role in cytoskeletal arrangement of cells, the secretion of insulin, clearing of glucose, and altered cell survival. Taken together, our novel findings suggest that DSG2 is an under-appreciated regulator of cell-cell interaction in the pancreatic islets, and thus poses as a potential molecular target to improve pancreatic islet transplantation to cure diabetes.
SYM-52-05

INTRACELLULAR AND SINGLE MOLECULAR INTRAVITAL MICROSCOPY TO STUDY ACTIN CYTOSKELETON ORGANISATION AND DYNAMICS IN LIVE MICE

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Current models of actin cytoskeleton organisation and function are primarily based on cell culture models. The actin cytoskeleton is exquisitely sensitive to the extracellular environment, thus it is imperative to study cytoskeletal dynamics in vivo, under physiological conditions. Intravital subcellular microscopy allows the visualisation and quantification of the formation of actin structures with high temporal and spatial resolution. We used this model system to investigate the de novo assembly of actin, tropomyosins and non-muscle myosin II on secretory granules during exocytosis in mouse salivary gland. We observed that actin and tropomyosin filaments co-polymerise while myosin II is added to the assembly several seconds later. The temporal and spatial segregation of the cytoskeletal elements suggests that multiple actin populations are precisely orchestrated to build a functional scaffold that drives membrane remodelling during exocytosis. We have developed a single molecule intravital microscopy approach to quantify the molecular dynamics of cytoskeletal components at nanoscale resolution. To achieve this we combined a highly inclined and laminated optical sheet (HILO) microscopy, sparse, endogenous fluorescent labelling and single emitter localisation and tracking in live mice. We then quantified the binding durations of fluorescently tagged actin, tropomyosins and myosins and mapped them spatially over super-resolution molecular density distributions revealing finely resolved cellular structures. We observed differences in the binding duration and displacement of tropomyosin isoforms and myosin motors in vivo that point to distinct nature of their interactions with actin filaments. Single molecule intravital microscopy will help us understand how cytoskeletal proteins interact within heterogeneous micro-assembly and distinct subcellular structures within a physiological environment.

SYM-53-02

THE PTENP14-PKCδ-TK AXIS IN HEALTH AND DISEASE

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Elevated levels of growth-promoting receptors on the cell surface can contribute to oncogenesis and resistance to targeted therapy. We have found that the tyrosine phosphatase PTENP14 (also called Pez) limits the recycling of endocytosed receptors back to the cell surface. We have now identified the key substrate of PTENP14, PKCδ (Belle et al, 2015), that regulates receptor recycling and the upstream kinase that phosphorylates PKCδ. This role of PTENP14 is consistent with the finding that the de novo assembly of actin, tropomyosins and non-muscle myosin II on secretory granules during exocytosis in mouse salivary gland. We observed that actin and tropomyosin filaments co-polymerise while myosin II is added to the assembly several seconds later. The temporal and spatial segregation of the cytoskeletal elements suggests that multiple actin populations are precisely orchestrated to build a functional scaffold that drives membrane remodelling during exocytosis. We have developed a single molecule intravital microscopy approach to quantify the molecular dynamics of cytoskeletal components at nanoscale resolution. To achieve this we combined a highly inclined and laminated optical sheet (HILO) microscopy, sparse, endogenous fluorescent labelling and single emitter localisation and tracking in live mice. We then quantified the binding durations of fluorescently tagged actin, tropomyosins and myosins and mapped them spatially over super-resolution molecular density distributions revealing finely resolved cellular structures. We observed differences in the binding duration and displacement of tropomyosin isoforms and myosin motors in vivo that point to distinct nature of their interactions with actin filaments. Single molecule intravital microscopy will help us understand how cytoskeletal proteins interact within heterogeneous micro-assembly and distinct subcellular structures within a physiological environment.

SYM-53-03

EUKARYOTIC ELONGATION FACTOR 2 KINASE PROMOTES THE SURVIVAL, MIGRATION AND INVASION OF CANCER CELLS

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The elongation stage of protein synthesis is one of the most energy- and nutrient-demanding processes in the cell. Control of translation elongation can differentially affect the synthesis of different proteins. The atypical protein kinase eEF2K (eukaryotic elongation factor-2 kinase) phosphorylates and inhibits eEF2, the protein which mediates elongation of ribosomes along mRNAs during elongation, thereby slowing down protein synthesis and reducing use of energy and amino acids. eEF2K is controlled by signalling pathways that detect cellular nutrient and energy levels. It is activated under various cellular stress conditions include nutrient starvation, energy insufficiency and hypoxia. Several lines of evidence, obtained using multiple cancer cell lines including glioma, medulloblastoma, colorectal, breast and lung cancer cells, show that eEF2K helps to protect such cells against starvation for nutrients such as glucose and/or amino acids. In contrast, under normal cell growth conditions, or in mice under vivarium conditions, eEF2K is not required. Disabling eEF2K affects the proteome of cancer cells, increasing or decreasing the levels of a number of proteins, presumably by altering their rates of synthesis. These include several proteins involved in cell migration, the cytoskeleton or related signalling pathways. Consistent with this, eEF2K promotes the migration of cancer cells and their ability to invade. eEF2K also promotes cancer cell migration in vivo. These data indicate that agents that inhibit eEF2K will prevent tumour growth and metastasis, without exerting adverse on-target side effects, identifying eEF2K as a potential target for novel anti-cancer therapeutics.

SYM-53-04

QUANTITATIVE N-TERMINOMICS AND PHOSPHOPROTEOMICS REVEAL DISTINCT SIGNALLING NETWORKS GOVERNING REGULATED NECROSIS OF NEURONS

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Excitotoxicity, initiated by over-stimulation of ionotropic glutamate receptors (iGluRs), is a major pathological process directing regulated necrosis of adult neurons in both acute and chronic neurological disorders. Upregulation of iGluRs allows massive influx of calcium ions into the affected neurons, leading to over-activation of two groups of neurotoxic calcium-dependent enzymes: (i) the cysteine proteases calpains, which catalyse limited proteolysis of specific neuronal proteins and (ii) neuronal nitric oxide synthase (nNOS), which generates excessive NO to induce oxidative damages. The calpain-proteolysed proteins and the NO-induced oxidative damages in turn modulate the activities of proteases, protein kinases and phosphatases to perturb the expression and phosphorylation of specific neuronal proteins. Presumably, these perturbed proteins form the signalling networks that direct neuronal necrosis. To define these neurotoxic signalling networks, we performed quantitative proteomics and biochemical analyses to identify the calpain substrates and the perturbed proteins in neurons undergoing excitotoxic cell death. Using the Terminal Amine Isotopic Labelling of Substrates (TAILS) proteomics method, we identified the exact sites of cleavage in ~300 neuronal proteins using the stable isotope dimethyl labelling method, we definitively identified over 150 neuronal proteins undergoing changes in abundance and/or phosphorylation levels at different time points after over-stimulation of iGluRs. Bioinformatic analysis predicted that these identified proteins form distinct signalling networks. Using biochemical approaches, we found that some components of the predicted signalling networks induce neuronal death by aberrant regulation of Erk1/2 and Akt, which are protein kinases critical to neuronal survival. Taken together, our findings illustrate how results of quantitative proteomic analyses can form the conceptual framework for investigation to define the molecular mechanism governing regulated necrosis of adult neurons.
SYM-53-04

PROTECTIVE FUNCTION FOR SITE-SPECIFIC PHOSPHORYLATION OF TAU BY MAP KINASE P38γ

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Site-specific protein phosphorylation is a central component of many signal transduction cascades. The neuronal tau protein is strongly phosphorylated, which is considered to contribute to toxicity induced by amyloid-β (Aβ) in Alzheimer’s disease (AD). In contrast to previous assumptions on tau phosphorylation, we found that site-specific phosphorylation of tau inhibited Aβ toxicity. This specific tau phosphorylation was mediated by neuronal p38 mitogen-activated protein (MAP) kinase p38γ in post-synaptic densities and interfered with postsynaptic excitotoxic signalling complexes engaged by Aβ. Accordingly, depletion of p38γ exacerbated neuronal circuit alterations, synaptic deficits, and premature death in a mouse model of AD, whereas increasing the activity of p38γ abolished these deficits. Furthermore, mimicking site-specific tau phosphorylation alleviated Aβ-induced neuronal death and offered protection from excitotoxicity. Consistently, newly generated CRISPR-engineered mice expressing phospho-mimicking variants of endogenous tau, were protected from acute excitotoxicity. Our work provides insights into postsynaptic signalling involving tau, describes an unprecedented function of MAP kinase p38γ and challenges a purely pathogenic role of tau phosphorylation in neuronal toxicity.

SYM-53-05

THE AUTOPHAGY INITIATOR ULK1 SENSITIZES AMPK TO ALLOSTERIC DRUGS

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AMP-activated protein kinase (AMPK) is a metabolic stress-sensing enzyme responsible for maintaining cellular energy homeostasis. Activation of AMPK by salicylate and the thienopyridone A-769662 is critically dependent on phosphorylation of Ser 108 in the β1 regulatory subunit. Here we show a possible role for Ser 108 phosphorylation in cell cycle regulation and promotion of pro-survival pathways in response to energy stress. We identify the autophagy initiator Unc-51-like kinase 1 (ULK1) as a β1-Ser 108 kinase in cells. Cellular β1-Ser 108 phosphorylation by ULK1 was dependent on AMPK β-subunit myristoylation, metabolic stress associated with elevated AMP/ATP ratio, and the intrinsic energy sensing capacity of AMPK; features consistent with an AMP-induced myristoyl switch mechanism. We further demonstrate cellular AMPK signalling independent of activation loop Thr 172 phosphorylation, providing potential insight into physiological roles for Ser 108 phosphorylation. These findings uncover new mechanisms by which AMPK could potentially maintain cellular energy homeostasis independently of Thr 172 phosphorylation.

SYM-54-01

WALL INGROWTH DEPOSITION IN PHLOEM PARENCHYMA TRANSFER CELLS OF ARABIDOPSIS IS A HETEROTRIMERIC TRAITS CONTROLLED BY THE MIR156/SPL MODULE

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Transfer cells (TCs) trans-differentiate from existing cell types to facilitate enhanced membrane transport of nutrients at symplasmic/apoplastic interfaces. The morphological specialization of TCs lies in their augmented surface area of plasma membrane resulting from intracellular wall ingrowths. In Arabidopsis, phloem parenchyma cell (PP) TCs form from PP cells in vascular bundles of cotyledons, leaves and sepals. We report that PP TCs with extensive wall ingrowths are ubiquitous in juvenile leaves, but substantially less abundant in adult leaves, an observation consistent with PP TC development representing a novel trait of heteroblasty or vegetative phase change (VPC) in Arabidopsis. Consistent with this conclusion, the abundance of PP TCs with extensive wall ingrowths varied across rosette development in three ecotypes that display different juvenile phase lengths, and extensive deposition of wall ingrowths was observed in rejuvenated leaves following defoliation. PP TC development across juvenile, transition and adult leaves correlated positively with levels of mir156, a major regulator of VPC in plants, and negatively with levels of mir156-targeted SQUAMOSA PROMOTER BINDING PROTEIN LIKE (SPL) genes. Corresponding changes in wall ingrowth deposition were observed when mir156 was overexpressed or its activity suppressed by target mimicry. Wall ingrowth deposition was reduced in plants carrying mir156-resistant forms of SPL9, SPL10 and SPL15, and was increased in the double mutant spl9/spl15. Importantly, no change in xylem abundance was observed in these lines, indicating that the VPC response of PP TCs was specific to altered wall ingrowth deposition. Collectively, our results point to wall ingrowth deposition in PP TCs being under control of the genetic program regulating VPC via the mir156/SPL module.

SYM-54-02

CELL WALL REGULATION AT THE TRANS-GOLGI NETWORK

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A polysaccharide-based cell wall surrounds all plant cells. The plant cell wall is flexible and constantly remodelled as the plant grows; new synthesis and deposition of cell wall material is critical for plant cell division, cell growth, and many types of cell differentiation. Biotic and abiotic factors can also influence cell wall deposition. Therefore, plants must monitor the status of the cell wall by perceiving developmental, abiotic and biotic signals and respond to these signals by controlling cell wall synthesis. We have identified members of a family of 7 transmembrane (7TM) domain containing proteins that are required for responses to cell wall stress. Consequently, cell wall stress, such as cellulose synthesis inhibition, leads to reduced growth, cell swelling, and reduced cellulose production in the 7tm mutants, compared to wild-type. A member of the 7TM family, 7TM1, can interact with components of the heterotrimeric G-protein complex, and G-protein complex mutants phenocopy the 7tm mutants during cellulose synthesis inhibition. Interestingly, 7TM1 localizes to the Golgi apparatus and the trans-Golgi network and is required for efficient trafficking of cellulose synthase enzymes to the plasma membrane. 7TM1 interaction with components of the G-protein complex also occurs in intracellular compartments. Together, these data suggest that the 7TM proteins are endomembrane localized putative G-protein coupled receptors that are required to modulate secretion of cell wall related components in response to cell wall signals.
SYMPOSIUM
THURSDAY

SYM-54-03
IDENTIFICATION OF NOVEL NUCLEOTIDE SUGAR TRANSPORTERS IN ARABIDOPSIS

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Plant cell walls are composed of polysaccharides, which with the exception of cellulose and callose, are synthesised in the Golgi apparatus by families of glycosyltransferases. The nucleotide sugar substrates necessary for the assembly of these polysaccharides are predominantly made in the cytosol. This subcellular partitioning of substrates and enzymes requires transmembrane transport into the Golgi lumen. Consequently, nucleotide sugar transporters (NSTs) have evolved to enable transport of nucleotide sugars from the cytosol into the Golgi and endoplasmic reticulum (ER) lumen. They belong to the NST/ triose phosphate translocator (TPT) superfamily and are found in all eukaryotes. Phylogenetic analyses have identified more than 50 members in Arabidopsis thaliana, which are distributed in six clades. Despite substantial efforts until 2013 few plant NSTs had been functionally characterised at the molecular level only accounting for the transport of GDP-Man, UDP-Gal, UDP-Glc and CMP-sialic acid, although the latter has not even been shown to be part of any plant polymer. Since this collection of nucleotide sugars only represents a small number of substrates, considering all glycosyltransferases involved in polysaccharide biosynthesis, there must be additional NSTs mediating the transport of key nucleotide sugars, such as UDP-Rha, UDP-GlcA, GDP-Fuc, UDP-Ara, UDP-Api, and CMP-Kdo which are crucial for cell wall biosynthesis. The functional characterisation of NSTs has been limited by the inadequate availability of substrates, especially radiolabelled substrates, and difficulties in conducting complementation studies in eukaryotic systems that show deficiency in the transport of nucleotide sugars that are important in plants. Consequently, we developed a novel biochemical approach that is not limited by the availability of radiolabelled substrates or the complexities of a genetic complementation. Using this approach, we rapidly screened the entire Arabidopsis NST family and subsequently identified almost all major NSTs required for proper wall biosynthesis.

SYM-54-04
ARABIDOPSIS CCC: A GOLGI AND TGN LOCALIZED ION TRANSPORTER WITH PUTATIVE ROLES IN OSMOREGULATION AND PROTEIN TRAFFICKING

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Cation-chloride cotransporters (CCC) mediate Na+:K+:Cl- symport, and localise to the trans-Golgi network (TGN). Most plants contain a single CCC in their genome. Strikingly, Arabidopsis ccc shows many defects, including low fertility, loss of apical dominance, reduced root and shoot biomass, dwarfism, and reduced primary root length. None of these phenotypes are yet connected to inorganic ion transport. Here, we further characterised ccc mutants, observing deformed leaves, failure to maintain the inflorescence meristem, abnormal root shape, defective initial root hair formation, reduced root hair length and more. How CCC influences these aspects of plant growth and development is unknown. CCC may be required for maintaining Golgi and TGN function, and protein trafficking. The protein composition of the plasma membrane (PM) might therefore be altered, especially for proteins undergoing frequent cycling to and from the PM, like auxin transporters or aquaporins; and the cell wall composition might be affected. Additionally, CCC may localise to the PM of some cells and contribute to active water transport, like some animal CCcs, thereby participating in root pressure and water supply to shoots. We are measuring root pressure and leaf water potential to investigate this, and assaying Xenopus oocyte cell volume changes. Yeast expression assays and stable plant transformation are being used to determine protein domains in CCC involved in transport or signalling. We are investigating cell patterning, using a PM-marker expressed in ccc plants, to visualise floral meristem organisation, leaf epidermal cells, and effects of changes in osmolality on roots. We aim to elucidate CCC function, and how this protein may influence plant fitness.

SYM-54-05
A CA2+-DEPENDENT REMODELLED ACTIN NETWORK DIRECTS VESICLE TRAFFICKING TO BUILD WALL INGROWTH PAPILLAE IN TRANSFER CELLS

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Transfer cell transport function is conferred by an enlarged plasma membrane area, enriched in nutrient transporters, supported on a scaffold of wall ingrowth papillae. Polarized plumes of elevated cytosolic Ca2+ define loci at which wall ingrowth papillae form in developing adaxial epidermal transfer cells of Vicia faba cotyledons induced to trans-differentiate on cotyledon transfer to culture. We evaluated the hypothesis that vesicle trafficking along a Ca2+-regulated remodelled actin network underpins this outcome. Polarized to the outer periclinal cytoplasm, a Ca2+-dependent remodelling of long actin bundles into short, thin bundle fragments was found to be essential for assembling wall ingrowth papillae but not the underlying uniform wall layer. The remodelled actin network directed polarized vesicle trafficking to sites of wall ingrowth papillae construction. A pharmacological study indicated that both exo- and endocytosis contributed to assembling wall ingrowth papillae. Potential candidates responsible for Ca2+-dependent actin remodelling, along with those identifying polarized exo- and endocytosis, were identified in a transcriptome RNA-seq database generated from the trans-differentiating epidermal cells. Of most significance, endocytosis was regulated by up-regulated expression of a dynamin-like isoform. How a cycle of localized exo- and endocytosis, regulated by Ca2+-dependent actin remodelling assemblies wall ingrowth papillae is discussed.

SYM-55-01
GENERATING HUMAN KIDNEY ORGANOIDs FROM PLURIPOTENT STEM CELLS

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The generation of complex tissue organoids via directed differentiation of human pluripotent stem cells brings the prospect of personalised drug testing, disease modelling and regenerative medicine. We have developed a protocol for the generation of kidney organoids comprised of nephrons, collecting duct, vasculature and surrounding interstitium (Takasato et al, Nature, 2015) from human pluripotent stem cells. This protocol relies upon the stepwise recapitulation of morphogenetic events previously characterised during normal kidney development in the mouse. The utility of the protocol for applications such as the modelling of human kidney disease will rely implicitly on the accuracy of this differentiation program, the reproducibility of the directed differentiation, the transferability between iPS lines, the functional authenticity of the human cell types generated and a capacity to scale up cell numbers. This presentation will discuss our progress towards all of these objectives.
INVESTIGATING ENVIRONMENTAL IMPACTS ON INTESTINAL STEM CELL FUNCTION USING ORGANOIDs

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The intestinal epithelium is a dynamic tissue that constantly renews via a small population of intestinal stem cells. This highly specialised monolayer acts to absorb nutrients as well as providing a barrier to pathogens and toxins present in the lumen of the intestinal tract. Despite a remarkable capacity to regenerate following injury, there are many degenerative diseases that manifest in the intestinal epithelial layer. In contrast, excess intestinal stem cells are a hallmark of overproliferative adenomas that can progress to the development of colorectal cancer. We are studying both the intrinsic and extrinsic factors that control the renewal, maintenance and differentiation of intestinal stem cells and how alterations in these processes contribute to disease states in both young and aged tissue. We are also investigating how environment factors including microbes and the toxins influence the epithelial layer. We utilise gastrointestinal organoid culture as a key technology for delineating these interactions and have established organoid models of disease states from mouse and human tissue.

RAPID, COMPLEX MODELS OF COLORECTAL CANCER: USING CRISPR/CAS9 GENOME EDITING TO TARGET FREQUENTLY ALTERED GENES

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In this age of next generation sequencing we are fast accruing more information on cancer associated genetic alterations than ever before. How do we translate this new knowledge into better outcomes for cancer patients? Clearly we must prioritise genetic alterations for study from this wealth of data. Here we utilise the organoid culture technique pioneered by the Clevers lab, combined with CRISPR/Cas9 genome editing technology to delineate these interactions and have established organoid models of disease states from mouse and human tissue.

HUMAN INDUCED PLURIPOTENT STEM CELL MODEL OF PCDH19-GIRLS CLUSTERING EPILEPSY REVEALS ROLES FOR THIS PROTOCADHERIN IN REGULATION OF NEURONAL POLARITY AND DIFFERENTIATION

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Human induced pluripotent stem cells (hiPSCs) provide a unique opportunity to study neurological disorders using disease relevant cells usually unattainable from the patients. We have generated hiPSCs from PCDH19-Girls Clustering Epilepsy (PCDH19-GCE) patient skin fibroblasts. PCDH19-GCE is a female specific epilepsy associated with a spectrum of neurodevelopmental and behavioural problems. It is caused by a variety of loss of function mutations in an X-chromosome gene, PCDH19, with 100s of cases reported to date. PCDH19-GCE is a disorder of cellular mosaics, that is females who undergo X-inactivation and males with somatic mosaicism (3 cases reported). In addition to generating PCDH19-GCE hiPSC we also developed an optimised protocol of cortical development based on a model of dual-SMAD inhibition, which we found reproducible across multiple PSC lines. Using this protocol and PCDH19-GCE hiPSCs, we modelled PCDH19-GCE by replicating the cellular mosaicism of the patient brain. We found that PCDH19 is important for the maintenance of neuronal cell polarity during cortical development, with loss-of-function mutation in PCDH19 being able to form neural rosettes, but unable to properly maintain these structures as evidenced by a 46% decrease in lumen size and a 63% decrease in the number of polarised structures/rosette area, compared to wildtype. A significant increase in the number of neurons at the edge of the rosettes was also observed suggesting increased neuronal differentiation. We also found that PCDH19 regulates axonal extensions with mutant neurons having a 60% increase in primary neurite length. Taken together this work identifies novel roles for PCDH19 in neuronal polarity during cortical development and neuronal differentiation and morphology.
SYM-56-01

INTRACELLULAR DELIVERY OF PROTEINS WITH PHYLOMERS - A NEW CLASS OF CELL PENETRATING PEPTIDES

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Cell penetrating peptides (CPPs) are very promising tools for the non-invasive delivery of therapeutic cargoes through the membrane and into the cell. However, the majority of CPPs identified to date show low efficacies in delivering biologics into the cytosol, as they often remain trapped within the endosomal compartment. This inefficiency has limited the clinical translation of compounds incorporating CPPs significantly. Phylogica’s Phylomers are a class of peptides derived from fragments of bio-diverse microbial genomes, that have been screened for new CPPs to improve delivery of macromolecules and peptides into cells using our endosome escape trap. We have developed a variety of functional assays to determine the extent of cytoplasmic delivery of these cargoes to the cytoplasm or nucleus. In some cases, these next generation Phylomer-derived CPPs achieved in vitro potencies in the nanomolar range and increased mouse survival of up to 20% in relevant cancer models. The above described screening platforms are now being applied in Phylogica’s internal discovery programs to identify Phylomers that target transcription factor oncoproteins such as c-MYC, n-MYC and STATS.

SYM-56-02

STRUCTURE-GUIDED DESIGN OF INSULIN ANALOGUES AND MIMETICS

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Insulin has been used therapeutically to treat diabetes since 1923. However, the molecular detail of the way in which it engages its target, the insulin receptor, has begun to emerge only recently. In this talk I will highlight the insights that these structural data provide and how they might be used to create a new generation of therapeutics to treat diabetes.

SYM-56-03

A FRAGMENT-BASED APPROACH TO TARGETING COENZYME A BIOSYNTHESIS IN BACTERIA

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Antibiotic resistance poses a serious threat to public health worldwide. There is a great need for new antibiotics with novel modes of action, that are not susceptible to pre-existing resistance mechanisms. Fragment-based approaches, which involve the identification of chemical fragments that bind to a target, and the subsequent structure-guided elaboration of these into high affinity binders, hold promise in this area of drug discovery where more traditional approaches have largely failed. In this study, a fragment-based approach was applied to develop novel inhibitors of a bacterial enzyme involved in the biosynthesis of coenzyme A (CoA), a fundamental enzyme cofactor utilized by as many as 9% of all enzymes. The target enzyme - dephospho-CoA kinase (DPCK) - catalyzes the final step in CoA biosynthesis, and has been demonstrated to be essential for the viability of multiple pathogenic bacteria. A library of fragments (molecules of low molecular weight and complexity) was screened against the Escherichia coli DPCK using two orthogonal biophysical techniques - differential scanning fluorimetry and ligand-observed NMR. The screen yielded weakly, but efficiently binding fragment hits with inhibitory activity. The dynamic behavior of the target presented a challenge for obtaining fragment co-crystal structures, and for this reason protein-observed NMR was used to validate and map the binding sites of fragment hits. One fragment hit was optimized to produce a series of higher affinity DPCK inhibitors, one representative of which yielded a co-crystal structure. The work has generated the first inhibitors of a DPCK from any organism.

SYM-56-04

BACTERIOCINS: POTENTIAL THERAPEUTICS TO ADDRESS ANTIBIOTIC RESISTANCE

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Antibiotic resistance is a growing threat to public health. Pathogens that are resistant to single or multiple drugs have emerged. Novel drugs are needed urgently to address this crisis. Extensive efforts have been performed over the past decades to discover new antimicrobial peptides (AMPs). One type of AMPs warranting attention is bacteriocins. Bacteriocins are ribosomally synthesized peptides of bacterial origin with either narrow- or broad spectrum antimicrobial activity. In this study, we report the discovery of a novel circular peptide, designated as plantacyclin B21AG, from Lactobacillus plantarum B21. Plantacyclin B21AG showed strong antimicrobial activity against food-borne pathogens including Clostridium perfringens and Listeria monocytogenes; food spoilage bacteria such as L. arabinosus; and other lactic acid bacteria. Whole genome sequencing revealed that the gene cluster responsible for the production, immunity and secretion of this peptide is located on a 20 kb native plasmid. Fast Protein Liquid Chromatography (FPLC) analysis showed a single peak at 214 nm. SDS-PAGE suggested a molecular weight of ~ 5.5 kDa and MALDI-TOF-MS measurements of 5668 Da. The amino acid sequence of plantacyclin B21AG was deduced by de novo peptide sequencing. A mass discrepancy between the theoretical and experimental masses of 18 Da strongly suggested that the bacteriocin is actually a circular peptide. Further study is currently being performed to solve the three-dimensional structure of plantacyclin B21AG. Analysis of the 3D structure coupled with functional studies will provide a better understanding of how bacteriocin recognises target bacteria, as informed strategies for the prevention of bacteria resistance in the future.
SYM-56-05

TRANSCRIPTOMICS FOR THE DISCOVERY OF NEW CLASSES OF VENOM PEPTIDE

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Animal venoms are complex mixtures of proteins, peptides, small organic molecules and salts. Venom components are often finely-tuned to alter, potentiate and selectively, the physiology of another organism. As such, venoms have been a valuable source of biomedical research tools, drug leads and drugs. I have been using transcriptomics combined with mass spectrometry–based proteomics to generate comprehensive portraits of venom composition for a range of venomous species. I will describe the use of these techniques in the discovery of several novel classes of venom peptide from marine cone snails. This includes a class of venom insulins, which are used by the marine snail Conus geographus to induce hypoglycaemia and “insulin shock” in their fish prey. These venom insulins have provided surprising new insight into the structure-activity of insulin, leading to the preclinical development of a new class of fast-acting insulin analogues.

SYM-57-01

EXTRACELLULAR VESICLE INVOLVEMENT IN NEURODEGENERATIVE DISEASES

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Neurodegenerative disorders such as Alzheimer’s (AD), Parkinson’s (PD) and prion diseases are associated with proteins that misfold and deposit in the brain. Many cell types, including neurons, release extracellular vesicles (EVs) which include microvesicles and exosomes. EVs have been shown to be involved in processing of proteins such as APP, α-synuclein, and PrP which are those involved in AD, PD and prion diseases respectively. Roles for these vesicles include cell-cell signalling, removal of unwanted proteins, and transfer of pathogens (including prion-like misfolded proteins) between cells. EVs contain distinct processed forms of these proteins and, in the case of prion disease, they contain the transmissible form of the misfolded prion protein. In addition to their protein content these vesicles have recently been shown to contain genetic material in the form of protein coding (mRNA) and noncoding RNA species. As exosomes can be isolated from circulating fluids such as serum, urine, and cerebrospinal fluid (CSF), they provide a potential source of protein and RNA biomarkers for neurological and other diseases.

SYM-57-02

AMNION EPITHELIAL CELL DERIVED EXOSOMES FOR IDIOPATHIC LUNG FIBROSIS - REGENERATIVE MEDICINE FOR A DEGENERATIVE DISEASE

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Background and rationale: The human amnion epithelial cells (hAECs) release extracellular vesicles (EVs) that appear to reflect the pro-regenerative properties of the hAECs themselves. A proteomics screen confirmed that hAEC-EVs package immunomodulatory molecules such as HLA-G, and ligands associated with stem cell niche maintenance, such as Wnt5A. RNA-Seq analysis revealed that 8 miRNAs with associated anti-fibrotic effects were amongst the most abundant transcripts. Aim: Given that lung fibrosis is though to be perpetuated by stem cell attrition, we sought to evaluate the potential of hAEC-EVs to reverse lung fibrosis in aged mice. Methods: Eleven-to-twelve month old female C57Bl6 mice (n=6 per group) were challenged with bleomycin (0.15U/kg) and fibrosis allowed to develop. On the 14th day post challenge, either 10g EVs resuspended in saline, or vehicle alone, was instilled intranasally. Mice were culled 28 days post challenge. Tissues were collected for histological analysis and endogenous lung stem cells (bronchioalveolar stem cells; CD31-, CD45-, EpCAM+, Sca1hi) were flow sorted for gene expression analysis using the Fluidigm Biomark HD. Results: The hAEC-EVs were well-tolerated, EpCAM+, Sca1hi) were flow sorted for gene expression analysis using the Fluidigm Biomark HD. Results: The hAEC-EVs were well-tolerated, with no morbidity or mortality associated with the intervention. Indeed, the hAEC-EVs reversed fibrosis to levels comparable to healthy controls and this was associated with a 50% reduction in myofibroblast activation (p<0.01). Coincident with these findings, we observed significant increase in the transcription of β-catenin, BMP4, Cyclin D1, FoxM1, NFATc1 and Sca-1 in the bronchioalveolar stem cells. When 3D organoids of the bronchioalveolar stem cells were cultured in the presence of the hAEC-EVs, we also observed a greater number of colonies (p<0.0001) where the average size of each colony type (alveolar, bronchiolar and mixed) was also greater than control organoids (p<0.01). Conclusion: hAEC-EVs exert potent anti-fibrotic effects when delivered intranasally to aged mice challenged with bleomycin. This appears to be associated with their ability to either activate endogenous stem cells within the lung or protect the stem cell niche during the injurious process.

SYM-57-03

THE LAST DANCE OF A DYING CELL

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Apoptosis occurs in essentially all tissues as part of development, homeostasis, and pathogenic processes including chronic inflammation and infection. During apoptosis, dying cells can disassemble into smaller membrane-bound vesicles called apoptotic bodies. Under certain conditions, cellular materials such as cytokines, cell surface molecules and microRNA can be packaged into apoptotic bodies as a mechanism to regulate immunity and tissue repair. Since billions of cells undergo apoptosis daily, the importance of apoptotic cell disassembly for health and disease is fundamental, yet the mechanisms involved in the formation of apoptotic bodies are poorly understood. Here, we describe a new mechanism of cell disassembly by apoptotic cells via the formation of a novel membrane protrusion called apoptopodia. Mechanistically, we have identified pannexin 1 membrane channels as a key regulator of apoptotic cell disassembly. Functionally, the formation of apoptotic bodies via apoptopodia could play an important role in cell clearance and viral infections. Additionally, we have also identified a novel selection of drugs that can modulate apoptotic body formation. Understanding the mechanistic basis and functional significance of this process will generate fundamental knowledge of the downstream consequence of cell death.
SYM-57-04
EXTRACELLULAR VESICLES REVEAL THE COMPLEXITY OF CANCER MULTIDRUG RESISTANCE

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Cancer multidrug resistance (MDR) occurs when cancer cells become cross-resistant to structurally and functionally unrelated drugs used in treatment. The overexpression of plasma membrane proteins belonging to the ABC superfamily of membrane transporters are synonyms with this phenotype. Typical members include P-glycoprotein (P-gp/ABCB1) and Multidrug Resistance Protein 1 (MRP-1/ABCC1). These transporters act to efflux drugs out from the plasma membrane of cancer cells by virtue of ATP hydrolysis, effectively conferring an intrinsic drug resistance to the cells. Our recent studies demonstrate that extracellular vesicles, specifically, microparticles (MPs), provide a novel pathway(s) for the dissemination and acquisition of MDR in cancer. This occurs through the intercellular transfer of functional resistance proteins, functional nucleic acids and through a capacity for active and passive drug sequestration. We have also shown that MPs derived from MDR cells readily confer the donor cell traits within recipient cancer cell populations, including MDR, enhanced metastatic capacity and altered tissue bioenergetic properties. Our most recent studies demonstrate the presence of a distinct and parallel pathway supporting the survival of MDR cancer cells also through immunological privilege. These findings provide the necessary basis for the design of novel therapeutic strategies, targeted at the prevention and circumvention of MDR clinically. This work was supported by research funds from the Cancer Council NSW (Grant RG-09-02), National Health and Medical Research Council, Australia (Project Grant APP1007613) and University of Technology Sydney to M.Bebawy. 1Head, Cancer Cell Biology and Therapeutics. Discipline of Pharmacy, Graduate School of Health, University of Technology, Sydney, NSW, Australia.

SYM-57-05
PHYSICAL COHERENCE AND NETWORK ANALYSIS REVEALS NEDD4 AS NOVEL REGULATOR OF EXOSOMAL BIOGENESIS

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Exosomes are small membrane extracellular vesicles that are secreted under physiological and pathological conditions. However, very little is known about the biogenesis of exosomes. Here, we studied ESCRT dependent mechanism of exosomal biogenesis by physical coherence and network based analysis. Using a conserved interaction network and physical coherence model, we intended to identify novel regulators of exosome secretion. A total of more than 50 proteins including NEDD4, SDCBP and STAMBP with significant p-values were identified. SDCBP has already been implicated in exosomal biogenesis hence validates our approach. Next, we evaluated the role of NEDD4 and STAMBP in exosomal biogenesis by molecular biology and biochemical experiments. In this study, we investigated the role of the NEDD4 in the biogenesis of exosomes. To address this, we generated CRISPR based NEDD4 knock-out (KO) in LIM1215 colorectal cancer cells. Exosome were isolated from wild type (WT) and KO cells using differential centrifugation coupled with ultracentrifugation. The isolated exosomes were quantified based on nanoparticle tracking analysis (NTA). The analysis revealed significant reduction in exosome secretion in NEDD4 KO cells compared to WT cells suggesting that NEDD4 is a novel regulator of exosomal biogenesis. Furthermore, immunoblotting was performed to confirm the reduced levels of exosomal enriched markers such as Alix. Follow up quantitative proteomic analysis of exosomes derived from WT/KO cells revealed protein cargo dependent on NEDD4. Hence, the results validate the predictions based on physical coherence and network models. Overall, in this study we have identified novel regulators of exosomal biogenesis using an integrated bioinformatics and experimental approach.

SYM-58-01
DOES PARKINSON’S DISEASE REALLY INVOLVE CYTOPATHOLOGICAL MITOCHONDRIAL DYSFUNCTION? A TALE TOLD BY TWO ORGANISMS - SOCIAL AMOEBAE AND HUMANS

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Parkinson's disease (PD) is the 2nd most common neurodegenerative disease, being increasing in prevalence as the population ages, and currently affects an estimated 1 in 350 Australians at a total economic cost of more than $10 billion per annum. About 10% of PD cases are familial in origin and associated with mutations in a score of genes. This has presented the opportunity to study the cytopathological effects of these mutations in simple model systems, including the cellular slime mould or social amoeba, Dictyostelium discoideum. It is widely believed that mitochondrial respiratory defects contribute to the cellular abnormalities in PD and Dictyostelium is a well-characterized model for mitochondrial disease. Exploiting this, we created several different Dictyostelium genetic models for PD and investigated whether these defects plays a role in the phenotypic outcomes. Neither the phenotypes of our PD models nor direct measures of mitochondrial function using state-of-the-art respirometry revealed impairment of mitochondrial respiratory function. Instead, mitochondrial OXPHOS complexes in Dictyostelium PD cells are functionally normal and active either at normal levels or at elevated levels. To determine if these findings are echoed in human cells, we conducted parallel studies of immortalized lymphocytes from human patients with idiopathic PD (IPD). The results showed that IPD cells have functionally normal OXPHOS complexes that are expressed at higher levels, supporting elevated mitochondrial respiration and correspondingly increased production of reactive O2 species (ROS). Cultured human neuroblastoma cells also exhibited mitochondrial hyperactivity after uptake of α-synuclein fibrils (but not monomers). It seems likely that elevated mitochondrial respiration in PD cells is a compensatory response to higher ATP demand, possibly resulting from defects in intracellular trafficking. Reported mitochondrial defects in post-mortem PD brains could be a secondary consequence of oxidative ROS damage accumulating over the long life time of neurons compared to other cell types.

SYM-58-02
USING RAPID AGEING ANIMALS TO EXPLORE DOPAMINERGIC CELL DEGENERATION

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Despite the prevalence of Parkinson's disease amongst our ageing population, an effective treatment beyond alleviating symptoms remains elusive. A limited understanding of the molecular basis of the disease has restricted the development of therapeutic strategies that directly target the major pathological feature of Parkinson's disease: the death of dopaminergic neurons. Elevated brain iron has long been known to occur in Parkinson's disease. An iron-dopamine redox couple in the brain has been proposed as an initiating chemical reaction preceding neuron death. Understanding the details of this damaging interaction may reveal how these two redox-active factors can be targeted through novel therapeutic intervention. To further understand this interaction we are using the simplified dopaminergic network of the nematode, Caenorhabditis elegans, to explore how and why dopaminergic neurons die. We have developed age-dependent models to explore the degenerative changes in dopaminergic cells following manipulation of iron homeostasis, endogenous dopamine synthesis and transport. Our data is consistent with alterations in iron homeostasis changing dopamine signalling and toxic interactions between dopamine and iron. This interaction also appears to be amenable for targeted intervention. Our research highlights that rapid ageing animal models are well suited for exploring neurodegenerative mechanisms and may ultimately identify new therapeutic opportunities.
**SYMPOSIA**

**SYM-58-03**

**MAKE DO AND MAKE NEW: HOW ZEBRAFISH RAPIDLY REGENERATES CNS INJURY**

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Zebrafish has a remarkable capacity to morphologically and functionally regenerate parts of its brain and spinal cord. The cellular and molecular mechanisms regulating neural regeneration are largely unknown. We have used in vivo imaging and drug screening in a zebrafish spinal cord and brain injury models to pin-point specific cells and signals that control regeneration. Surprisingly, we identified two temporally and mechanistically distinct waves of cellular regeneration in the spinal cord. The initial wave of regeneration relies on cell migration of pre-existing neuronal progenitors to the lesion site and that enables rapid functional recovery. The second wave of regeneration involves activation of quiescent neural stem cells and regenerative neurogenesis. Remarkably, the cell production compensates for lost tissue at injury site as well as the cells depleted from proximal areas via migration. The two waves of regeneration deotrate how the zebrafish are able to rapidly regain motor function within days after complete ablation but also replenish lost tissue over time. The spinal cord consists of diverse glial and neural cell types that are precisely organized. How the neural architecture and divergent cells are restored after injury? We identified that ependymal cells act as neural stem cells after injury. The ependymal cells maintain distinct positional codes that are inherited to asymmetically produce amplifying progenitors that in turn migrate to lesion site. Consequently the regeneration of specific sub-types of cells is controlled at stem cell level. Furthermore, we have identified molecules and pathways that stimulate neural regeneration in the spinal cord. Activation of such pathways in mammals may be relevant for future therapies since the key cell types enabling neural and axonal regeneration in zebrafish have direct counterparts in mammals.

**SYM-58-04**

**NON-SELF MUTATION: DOUBLE-STRANDED RNA ELICITS ANTIVIRAL CELL DEATH RESPONSE IN A DROSOPHILA MODEL OF EXPANDED REPEAT NEOURODEGENERATIVE DISEASES**

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About 20 dominantly inherited expanded repeat diseases typically share the symptom of neurodegeneration. The expanded repeats are found in diverse locations in unrelated genes and comprise various repeat length and sequence composition. Either they have commonalities in their pathogenesis or there are a very large number of pathways that cause neurodegeneration. All repeats are transcriptionally raising the possibility that RNA is a common causal agent. We have identified a novel mutation mechanism whereby an endogenous mutant gene product is no longer recognised as ‘self’. Cellular RNA is usually modified in a manner that enables it to avoid being recognised by RNA-binding Pattern Recognition Receptors (PRRs). Expanded CAG·CUG repeat double stranded RNA causes cell death when expressed in Drosophila model of neurodegenerative disease. The double stranded repeat RNA is recognised by dicer2 as a foreign or non-self molecule. Drosophila dicer2 is a member of the RIG-I-like PRRs and in this case acts in this capacity (as neither R2D2 or loquacious, the cofactors for its RNAi pathway, are necessary for toxicity). When elevated levels of the RNA-editing enzyme ADA1 are present toxicity is diminished as ADA1 modifies the CAS to CIG confirming ‘self’ status. When genes involved in the innate inflammatory response pathway are reduced in expression pathogenesis is diminished. In addition, elevated levels of TNF and anti-microbial peptide (drosomycin) are produced indicating activation of the inflammatory response. This ‘non-self’ endogenous RNA mechanism also provides a plausible explanation for the finding that human endogenous retrovirus-K contributes to motor neuron disease. References a. Samaraweera, S. et al., (2013) Hum. Mol. Genet. 22, 2811–9. b. Li, W., et al., (2015) Sci Transl Med. 7(307): 307ra153.

**SYM-58-05**

**ALS-LINKED MUTATIONS IN UBQLN2 CAUSE ER STRESS AND IMPAIR AUTOPHAGY FUNCTION**

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Amyotrophic lateral sclerosis (ALS) is a rapidly progressive, fatal neurodegenerative disease characterised by the death of upper and lower motor neurons. Ubiquilin-2 (UBQLN2) mutations are associated with X-linked juvenile and adult onset inherited ALS and ALS/dementia. UBQLN2 is involved in protein degradation through the ubiquitin-proteasome system (UPS) and autophagy. Transgenic rats expressing an ALS-linked UBQLN2 mutation (UBQLN2<sup>2047<sup>Δ</sup></sup>) display perturbations in ER and Golgi morphology. However, the effect of mutant UBQLN2 on related cellular pathogenic mechanisms linked to ALS remain unclear. The aim of this study was to investigate the effect of UBQLN2 mutations on ER stress, ER–Golgi transport and autophagy. Mouse neuroblastoma Neuro2a cells co-expressing wild type or ALS-linked mutant UBQLN2 (UBQLN2<sup>2047<sup>Δ</sup></sup>, UBQLN2<sup>2067<sup>Δ</sup></sup>) with mCherry tagged temperature sensitive mutant vesicular stomatitis virus glycoprotein (VSVG<sup>ΔS548</sup>) or tandem mCherry-EGFP-LC2 were examined for ER–Golgi trafficking and autophagy respectively. In addition, ER stress was examined in these cells using immunocytochemistry for ER stress markers CHOP and XBP-1. These studies revealed that similar to other mutant proteins linked to ALS, mutant UBQLN2 inhibited transport between the ER and Golgi compartments, and triggered ER stress. Similarly, cells expressing mutant UBQLN2 display significant accumulation of LC3-I, implying dysregulation of the autophagic machinery. Consistent with this notion, we also detected more vesicular structure co-localized with LC3 in mutant UBQLN2 expressing cells compared to wild type or untransfected cells. This study therefore identifies disease mechanisms triggered by mutant UBQLN2 in ALS. Inhibition of essential regulatory functions of ER–Golgi transport and autophagy are disrupted, triggering ER stress, which accelerates the accumulation of toxic aggregation-prone proteins. This study therefore provides novel insights into the importance of UBQLN2 in the pathogenesis of ALS.

**SYM-59-01**

**UNDERSTANDING SPHINGOLIPID MEDIATORS OF INSULIN RESISTANCE**

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Ectopic lipid accumulation in non-adipose tissues is thought to be a major cause of impaired glucose metabolism and insulin resistance in obesity and type 2 diabetes. However, there is accumulating evidence showing that not all lipids are equal in this respect. In a recent comparison of five common inbred mouse strains, we observed a close association between differential ceramide acyl composition in tissues and the development of metabolic dysfunction. Acylation of ceramides is regulated by the family of ceramide synthase enzymes, isoforms of which have distinct preferences for specific acyl-CoA substrates. Here I will discuss our studies using genetic and pharmacological approaches to examine the metabolic effects of targeting different ceramide synthase isoforms.
**SYM-59-03**

**THE METABOLIC CONSEQUENCES OF FECAL MICROBIOTA TRANSPLANTATION (FMT) IN MICE**

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Background: The gastrointestinal microbiota is a community of microorganisms that reside in the digestive tract. Studies have suggested that the microbiota composition may contribute to the development of obesity and the metabolic syndrome. Exercise has been shown to alter the microbiota composition by increasing diversity and altering specific bacteria species. We tested whether fecal microbiota transplantation (FMT) from exercise-trained mice to recipient mice alters body composition and metabolism. Methods: C57BL6/J mice were fed a Chow or high fat diet (HFD) for 4-weeks to induce obesity and insulin resistance. Mice were further divided into sedentary or exercise training groups (treadmill training for 6-weeks) while maintaining the respective diets (four groups of donor mice: Chow sedentary or exercise and HFD sedentary or exercised). Recipient mice were inoculated via oral gavage with the faeces from the respective donor groups once a week for 6-weeks and body composition and metabolism assessed. Results: While the HFD led to glucose intolerance and obesity, exercise training resulted in a small decrease in body fat and improved glucose tolerance. FMT from the donor groups did not alter body composition (weight, fat mass, lean mass) in any of the recipient groups. Unexpectedly, given the lack of an effect on adiposity, glucose intolerance was disrupted in the mice inoculated with faeces derived from mice on a HFD irrespective of exercise status and this was associated with a decrease in insulin-stimulated glucose clearance into white adipose tissue and the large intestine. Conclusion: FMT can transmit HFD-induced aspects of disrupted glucose metabolism to recipient mice independently of any change in adiposity. However, FMT from exercise-trained donor mice appears to elicit beneficial effects. Disclosure: No conflict of interest.

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**SYM-59-05**

**INSULIN RECEPTOR SUBSTRATE-ASSOCIATED PROTEINS: THE KEYS TO REGULATION OF INSULIN-LIKE ACTIONS**

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Insulin-like peptides, such as insulin-like growth factors (IGFs) and insulin, induce a variety of bioactivities, such as growth, differentiation, survival, increased anabolism, and decreased catabolism in many cell types and in vivo. In general, IGFs or insulin bind to IGF-1 receptor (IGF-IR) or insulin receptor (IR), activating the receptor tyrosine kinase. Insulin receptor substrates (IRSs) are known to be major substrates of receptor kinases, mediating IGF/insulin signals to direct bioactivities. Recently, we discovered that IRSs form high-molecular-mass complexes (referred to here as IRSomes) even without IGF/insulin stimulation. These complexes contain proteins (referred to here as IRSAPs; IRS-associated proteins), which modulate tyrosine phosphorylation of IRSs by receptor kinases, control IRS stability, and determine intracellular localization of IRSs. We found that ubiquitin ligase Nedd4 associates with IRS-2 and Nedd4 conjugates mono-ubiquitin to the IRS-2 C-terminal region. Ubiquitinated IRS-2 is in turn recognized by Eps15, which possibly recruited IRS-2 to plasma membrane. Consequently, IGF-induced tyrosine phosphorylation of IRS-2 is enhanced by IGF-1 receptor kinase, which leads to the augmentation of IGF signals and mitogenic activities. Taking other data together, our study demonstrated that this novel mechanism plays important roles to induce somatic growth as well as cancer growth in response to IGFs.