PLENARY LECTURES

Monday – Thursday
High resolution structural analysis of macromolecular assemblies and cellular architectures is essential for understanding the mechanisms of biological functions because they are all determined by the structures and dynamics of the molecules and cells at atomic level. Although X-ray crystallography and NMR are powerful tools for structural analysis of macromolecules, the difficulty in crystallization and the size limit of target molecules pause severe limitations on the application of the former and the latter, respectively. Filamentous protein complexes are particularly difficult target because the length is not uniform. Electron cryomicroscopy (cryoEM) and image analysis is a powerful tool because there is no size limitation and it requires only a very small amount of sample for data collection. The achievable resolution, however, tends to be limited by intrinsically low signal to noise ratio (S/N) of cryoEM images due to extremely low electron dose to avoid radiation damage. Therefore, tens of thousands of particle images have to be collected, classified according to the orientation, aligned and averaged to reconstruct high-resolution 3D images. However, accurate image alignment is difficult due to the intrinsically low S/N of cryoEM images. We developed techniques to improve the image S/N and speed of image collection by the use of an energy filter and CCD camera in combination with the liquid helium-cooled specimen stage on an electron microscope with a field emission electron gun operated at 200 - 300 kV. We have gained nearly 5 times higher S/N than before by controlling ice thickness and raising the specimen temperature from 4 K to around 50 K. The undesirable charging effect has also been minimized at 50 K, allowing us to use nearly all the collected images for analysis, while only a few % of images were usable at 4 K. Single particle image analysis now allows the high-resolution structural analysis of filamentous protein complexes to be completed within a week, and the achievable resolution is beyond 4 Å. I will describe the structures of various filamentous protein complexes and discuss the potential of the method for future life sciences.
PLE-MON-02

RE-DESIGNING PHOTOSYNTHESIS TO INCREASE YIELD - SYNTHETIC APPROACHES AND NATURAL VARIATION

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The primary determinant of crop yield is the cumulative rate of photosynthesis over the growing season which is the result of the crop’s ability to capture light, the efficiency by which this light is converted to biomass and how much biomass is converted into the usable product e.g. grain in the case of wheat and rice. Photosynthesis has never been used as a parameter to select for high yielding varieties and often the rate of photosynthesis per unit leaf area shows a negative correlation with yield. However, there is now compelling evidence from transgenic studies that the manipulation of targeted steps in the photosynthetic process has the potential to increase crop yield and maximise production. Furthermore, there is significant natural variation in photosynthetic performance within and between species, the exploitation of which to improve photosynthesis is largely unexplored. Given that the increasing world population is putting ever greater demands on agriculture to provide high yielding crop plants for food and fuel, it is essential that the potential of improving photosynthesis for yield increases is fully exploited. In this talk I will review the evidence to support this approach to increasing yield using synthetic biology based technology and will also explore the potential for exploitation of existing natural variation. Raines CA (2011) Plant Phys 155, 36-42; Lawson et al (2012) COB 23, 215-220.
AMP-ACTIVATED PROTEIN KINASE: A CELLULAR ENERGY SENSOR WITH NEW ROLES IN CANCER AND INFLAMMATION

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The AMP-activated protein kinase (AMPK) occurs as heterotrimeric complexes comprising a catalytic α subunit and regulatory β and γ subunits. It was discovered via its ability to phosphorylate and activate enzymes that catalyze regulatory steps in lipid synthesis, but it is now clear that it acts as a central sensor of cellular energy that has tens, if not hundreds, of downstream targets. AMPK is activated by falling cellular energy status via a complex mechanism involving allosteric activation by AMP, and modulation of the phosphorylation/dephosphorylation of Thr172 (within the α subunit) by upstream kinases and phosphatases. One activated, AMPK attempts to restore cellular energy homeostasis by activating catabolic pathways that generate ATP, while switching off biosynthetic pathways and other processes (including progress through the cell cycle) that consume ATP. The discovery that the major kinase phosphorylating Thr172 was a complex containing the tumour suppressor LKB1 provided a link between AMPK and cancer, and suggested that AMPK might exert the tumour suppressor functions of LKB1. Most rapidly proliferating cells, including tumour cells, utilize rapid glucose uptake and glycolysis to lactate to generate ATP, rather than the more energy-efficient mitochondrial oxidative metabolism. This phenomenon (the Warburg effect) may occur in part because the TCA cycle is being used not simply for catabolic purposes but also for provision of biosynthetic precursors, particularly citrate for lipid biosynthesis. Budding yeast display a related phenomenon, in that when incubated in high glucose they grow rapidly using fermentation (glycolysis to ethanol) to generate their ATP, whereas when glucose runs low their growth slows and they switch to use of oxidative metabolism instead. Intriguingly, the yeast orthologue of AMPK (the SNF1 complex) is required for this metabolic switch. By promoting oxidative metabolism, AMPK exerts a similar “anti-Warburg” effect in human cells. Thus, by inhibiting progress through the cell cycle and by promoting the metabolism utilized by quiescent cells, AMPK would be expected to exert anti-cancer effects. This idea is supported by findings that the AMPK-activating drug metformin reduces the risk of cancer in diabetics. Cells involved in inflammation, including dendritic cells and M1 macrophages, also tend to display rapid glycolysis even under normoxic conditions (aerobic glycolysis), whereas cells involved in the resolution of inflammation (e.g. M2 macrophages) tend to utilize oxidative metabolism instead. By promoting the latter, AMPK would exert anti-inflammatory effects, an idea supported by our findings that salicylate (the major breakdown product of the anti-inflammatory drug aspirin) activates AMPK by direct binding to the α subunit. Recent findings that may be discussed in the lecture include the detailed mechanism by which adenine nucleotides regulate AMPK, the identity of the upstream phosphatases that dephosphorylate Thr172, and the mechanism by which the insulin-activated kinase Akt/PKB (which is activated in many tumour cells) down-regulates AMPK by phosphorylating a site within the “ST loop” in the α subunit, which then sterically hinders Thr172 phosphorylation.
INFLAMMATORY MEDIATORS AND FIBROSIS IN NORMAL AND DYSTROPHIC SKELETAL MUSCLE

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Effective repair of damaged tissues and organs requires the coordinated action of several cell types, including infiltrating inflammatory cells and resident cells. Recent findings have uncovered a central role for macrophages in the repair of skeletal muscle after acute damage. If damage persists, as in skeletal muscle pathologies, macrophage infiltration perpetuates and leads to progressive fibrosis, thus exacerbating disease severity. I will discuss how dynamic changes in macrophage populations and activation states in the damaged muscle tissue contribute to its efficient regeneration. Conversely, dysregulation of the inflammatory response may lead to aberrant muscle repair and fibrosis development, as in muscular dystrophy progression.
PLE-MON-05

“ALU”STRIOUS EFFECTS ON HUMAN RNA METABOLISM: POST-TRANSCRIPTIONAL GENE REGULATION BY INTER-AND INTRA-MOLECULAR ALU ELEMENT BASE-PAIRING

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Staufen1-mediated mRNA decay (SMD), which occurs when translation terminates sufficiently upstream of a STAU-binding site (SBS), is important for myogenesis, adipogenesis and many other developmental and homeostatic pathways\(^1\). An SBS can be created by intramolecular base-pairing within an mRNA 3’-untranslated region (3’UTR) or by intermolecular base-pairing between a 3’UTR and a long non-coding RNA (lncRNA), which we call a ½-sbsRNA. Intermolecular base-pairing in humans involves Alu elements, which are a type of small interspersed repetitive element (SINE)\(^2\), whereas intermolecular base-pairing in rodents involves B and identifier (ID) SINEs\(^3\). Roles of STAU1 dimerization\(^4\) and the STAU1 paralog STAU2\(^5\) in SMD will be discussed. A new mechanism by which mRNAs crosstalk in a way that involves direct mRNA–mRNA interactions between 3’UTR Alu elements in each mRNA will also be described. SMD of each duplexed mRNA occurs provided each is translated; if only one mRNA is translated, then it alone is targeted for SMD, explaining why those ½-sbsRNAs shown to date to generate an SBS are not targeted for SMD. We demonstrate the importance of mRNA–mRNA-triggered SMD to the processes of cell migration and invasion in studies of the mRNA encoding CUB domain-containing protein 1 (CDCP1), which functions at the cell surface as an anti-apoptotic agent that promotes tumor-cell viability during or in early states of metastatic colonization\(^6\). Our findings uncover a previously unappreciated role for mammalian-cell mRNAs. This unexpected connection, together with our discovery of how STAU1 binding to inverted repeated 3’UTR Alu elements (IRA\(\text{Alu}\)s) competes with nuclear retention mediated by p54\(^{\text{nrb}}\) binding to 3’UTR IRA\(\text{Alu}\)s and also the repression of cytoplasmic translation mediated by protein kinase R (PKR) binding to 3’UTR IRA\(\text{Alu}\)s\(^7\), adds new and unanticipated layers of complexity to the intricate network of post-transcriptional interactions that regulate gene expression.

\(^{5}\text{Park E, Gleghorn ML, Maquat LE (2013) Staufen1-mediated mRNA decay by binding to its own sequence and its paralog and promoting UPF1 helicase but not ATPase activity. Proc Natl Acad Sci USA, 110: 405-12.}\)
\(^{7}\text{Elbarbary, R., Li, W., Tian, B. and Maquat, L.E. (2013) STAU1 binding 3’UTR IRA\(\text{Alu}\)s complements nuclear retention to protect cells from PKR-mediated translational shutdown. Genes & Dev. 27:1495-1510.}\)
HIGH-THROUGHPUT GENETIC SCREENS TO DEFINE MECHANISMS OF HUMAN NEURODEGENERATIVE DISEASES

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Protein folding is critically important for all life, from microbes to man. A bafflingly diverse set of cellular mechanisms has evolved to coordinate this exquisitely sensitive process. Not unexpectedly, problems in protein folding are the root cause of many of the most devastating diseases, which represent a major challenge to public health worldwide, especially as our population continues to age. Referred to collectively as protein-misfolding disorders, these truly disastrous neurodegenerative diseases include Alzheimer disease, Parkinson disease and amyotrophic lateral sclerosis (ALS or Lou Gehrig’s disease). Understanding, at a mechanistic level, the cellular consequences of protein misfolding will help to suggest potential strategies for therapeutic intervention. We use the baker’s yeast, *Saccharomyces cerevisiae*, as a model system to study the cell biology underpinning protein-misfolding diseases. Since dealing with misfolded proteins is an ancient problem, we hypothesize that fundamental mechanisms that we discover in yeast are likely to be conserved to man. Our long-term goal is to identify the critical genes and cellular pathways affected by misfolded human disease proteins. We have focused on the ALS disease proteins TDP-43 and FUS/TLS and have used yeast models to define novel disease mechanisms and have extended our findings into animal models as well as human patient samples. Remarkably, we recently discovered mutations in one of the human homologs of a hit from our yeast TDP-43 modifier screen in ALS patients. Mutations in this gene are relatively common (~5% of cases) and might be one of the most common genetic risk factor for ALS discovered to date. This underscores the power of such simple model systems to discover novel and unexpected insight into human disease. Launching from these studies in yeast to test known ALS disease genes, we have also been using yeast as a discovery platform to predict novel ALS disease genes based on functional properties and to combine this approach with human genetics and next generation sequencing to further define the complex genetic landscape of ALS. We anticipate that our novel approach will be broadly applicable to other human disease situations, many of which are deeply rooted in basic biology. Finally, our yeast TDP-43 genetic modifier screens have recently led to the discovery of a completely unexpected and novel therapeutic target for ALS. We found that inhibiting the RNA lariat debranching enzyme, Dbr1, potently protects against TDP-43 cytoplasmic aggregation and toxicity in multiple model systems. Thus, it seems that our approach to use yeast cells and genetic screens to study mechanisms of human neurodegenerative diseases has provided novel insight in two major ways: 1) discovery of a common genetic risk factors for ALS and 2) discovery of a novel and unexpected candidate therapeutic target for ALS.
FOLIAR WATER UPTAKE AND WATER RELATIONS OF TREES IN TROPICAL CLOUD FORESTS: RECENT INSIGHTS AND FUTURE PERSPECTIVES

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Tropical Montane Cloud Forests (TMCF) represent an important repository of biodiversity and are characterized climatically by receiving frequent fog events. These forests also play an important role in the regional water cycle because of its ability to intercept and harvest significant amounts of water from orographic clouds and mist. TMCF are considered one of the most vulnerable ecosystems to climate change and several climatic models predict drier atmospheric conditions for high-altitude tropical regions due to an increase in the mean altitude of formation of the orographic cloud layer that originates fog. To evaluate the impact of predicted changes in climate in the distribution of species, we must have a better understanding of how fog affects the water relations and ecological performance of TMCF plants. In this talk, I will provide an overview of recent findings on the water relations of cloud forest species, focusing on the diversity of tree responses to drought and the prevalence and ecophysiological consequences of foliar water uptake (FWU). To reach these goals, we are undertaking a number of greenhouse and field experiments using multiple methods, including stable isotopes tracer measurements, analysis of high resolution microclimatic data (from a station installed at 2000 m in the middle of the fog belt), monitoring of tree water use using sapflow, leaf gas exchange and xylem hydraulics under contrasting climatic conditions. We have found that many species can absorb fog water directly through their leaves. Fog can significantly reduce tree transpiration, cause reversals in sapflow direction and be transported towards the soil through the xylem. This novel finding on water transport through plants challenges the current unidirectional soil-plant-atmosphere (SPAC) model. Foliar water uptake of fog might be especially important in rainless periods as it alleviates tree water stress and favors the maintenance of positive carbon balance for TMCF trees, promoting growth and increasing survival. Collectively, our results suggest that fog is a key water source for TCMF trees and may help explain the persistence of species and maintenance of important ecosystem services in tropical montane cloud forests.
PLE-TUE-08

STRUCTURAL INSIGHTS INTO ACTIN FILAMENT DISASSEMBLY FROM THE MALARIA PARASITE

Wong W.1, 2, Webb A.I.1, 2, Olshina M.A.1, 2, Hanssen E.2, Catimel B.3, Suarez C.4, Nebi T.1, 2, Kovar D.R.4 and Baum J.1, 2
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Actin depolymerizing factor (ADF)/cofilins are essential regulators of filamentous (F)-actin turnover across eukaryotic cells, from single celled protists through to metazoa. These multifunctional proteins facilitate both the stabilization and severing of F-actin in a concentration-dependent manner. At high concentrations ADF/cofilins bind stably to F-actin longitudinally between two adjacent actin protomers forming a decorative interaction, stabilising the filament. Low-densities of ADF/cofilins, in contrast, result in optimal F-actin severing, disassembling the filament. To date, how a single protein achieves these contrasting modalities is not certain. Current theoretical models favour the scenario where a single mode of interaction (decoration) can lead to either stability or severing based on a complex interpretation of biophysical forces. Work in the Baum laboratory over the last few years has focussed on understanding actin regulators in the malaria parasite, the ADF/cofilin proteins in particular. Uniquely, the malaria parasite ADF/cofilin PfADF1 can sever actin filaments but cannot stably bind to them. This suggests the possibility that, contrary to accepted dogma, ADF/cofilin modes of function (severing and decoration) may not be based on the same single mode of interaction. Towards exploring the biochemical basis of actin filament severing by ADF/cofilins, we have used chemical cross-linking and mass spectrometry (XL-MS) combined with protein structure reconstruction to explore the proximate amino acids between actin and ADF/cofilins from divergent species (malaria parasite and human) at near atomic resolution. Given the ability of XL-MS to reveal transient protein complexes that are not possible with techniques biased towards stable complexes, such as cryoelectron microscopy, we can reveal for the first time a conserved novel F-actin-binding interface (actin binding site 2), which can explain filament severing, independent of canonical filament decoration (via actin binding site 1). We can confirm the existence of this second site using complementary biochemical techniques and single molecule TIRF microscopy imaging of actin filament disassembly. Critically, the overlapping nature of the canonical and novel binding sites suggests a mechanism whereby undecorated F-actin regions expose the second binding site, thereby enabling severing. Thus, mutually exclusive binding interactions can explain the basis of actin filament decoration or severing, resolving the molecular basis for actin filament disassembly across eukaryotic cells.
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Chlorophylls of oxygenic photosynthesis are the most abundant biological pigments. Most of them absorb strongly in the visible spectral range of sunlight and have a relatively long-lived excited state to meet the excitation energy transfer and energy transduction requirements for photosynthesis. There are five different forms of chlorophylls. Chl a, b and c were identified in the 19th century; Chl d was reported in 1943, more than 70 years after the other chlorophylls. The fifth chlorophyll, Chl f, was reported 67 years after the previously discovered chlorophyll. These five chlorophylls show different absorption properties, i.e. they can harvest solar light at different wavelengths. The chemical structures of Chl d and Chl f are distinguished from Chl a by a formyl group replacement on ring A. The formyl substitution at C-3 results in a red shift to 696 nm in Chl d, that at C-2 in a red-shift to 706 nm in Chl f, giving them the name, red-shifted chlorophylls. The red-shifted absorbance allows these organisms to use light that cannot be used by other photosynthetic organisms. This has challenged the minimum threshold energy input for oxygenic photosynthesis. Organisms that contain Chl f can access light up to 760 nm, which is a new energy boundary for oxygenic photosynthesis — a significant 5% increase over Chl d containing organism. The red-shifted chlorophylls have propelled the field to re-evaluate the minimum threshold energy of oxygenic photosynthesis. One central question in understanding the occurrence of red-shifted chlorophylls is how this far red-shifts can be extended while still meeting the energy requirements for driving photosynthetic reactions? Photosynthetic organisms have modified the structures of chlorophylls in many ways. The photosynthetic function of novel chlorophylls will be discussed.
THE NEDD4 FAMILY OF UBIQUITIN-PROTEIN LIGASES

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Mouse *Nedd4* was cloned as one of the *Nedd* genes in 1992. The proteins encoded by some of the *Nedd* genes subsequently became prototypic members of the new functional groups. For example, Nedd8 turned out to be a protein that regulates other proteins when it is attached to them; a process now commonly termed “Neddylation”. Nedd2, now called caspase-2, became one of the first and most conserved of the apoptosis effector proteases known as caspases. Nedd4 became the prototype for a family of ubiquitin-protein ligases (“the Nedd4 family”) defined by a unique domain architecture. The Nedd4 family of ubiquitin-protein ligases contain an amino-terminal C2 domain, 2-4 WW domains and a carboxyl-terminal HECT domain. The C2 domain binds lipid and proteins, WW domains are protein-protein interaction domains which recruit substrates and regulators, whereas HECT is the catalytic domain that binds the ubiquitin conjugating enzyme (E2) to facilitate transfer of ubiquitin to the substrates. The Nedd4 family members play various essential functions in animal physiology, such as controlling growth, development of specific tissues, blood pressure, inflammation and iron homeostasis. I will discuss some of the recent work from my laboratory focusing on the biology and functional analyses of this important family of ubiquitin-protein ligases.
HORMONES AND PLANT DEVELOPMENT

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Forward genetics has proven to be a powerful tool for determining the role of plant hormones in the control of plant development. Thirty years ago not only were the biologically active molecules in many cases not known, their biosynthetic pathways were not fully understood and the prevailing view was that the levels of the hormones did not control developmental processes. Further, how a limited number of hormones could control a range of processes in different tissues was unclear. Through the use of a combination of mutants, precise analytical methods, detailed physiological experimentation and molecular techniques we now possess a better understanding of how plant hormone levels regulate processes such as stem elongation, photomorphogenesis, flowering, seed development, nodulation and mycorrhizal development. In collaboration with many excellent students, post-docs and colleagues our group has used peas as a model to explore these processes. Through analyses of dwarf mutants such as Mendel’s le and lke we have established GA, and castersterone as the major biologically active gibberellin and brassinosteroid, respectively, in peas. The slender mutant sln, which encodes a GA 2-oxidase responsible for de-activating GA, was the first mutation to show that deactivation pathways were critical in regulating the level of biologically active gibberellins. The tissue specificity of the na mutation gave the first indication that gibberellin levels were tightly regulated at the individual tissue level and the importance of gene families in this regulation. The lh mutation, which blocks ent-kaurene oxidase activity, demonstrated that biologically active GAs are essential for the early stages of seed development. More recently the use of mutants has demonstrated the potential roles of the gibberellins, auxins and strigolactones in the regulation of the development of nodulation and mycorrhizal symbioses. Recent work has also simplified our understanding of auxin biosynthesis, reducing the number of potential pathways from five, and showing that in pea seeds IAA and 4-Cl-IAA are produced predominantly through parallel pathways via indole-3-pyruvic acid and 4-Cl-indole-3-pyruvic acid, respectively.
THE ACTIVE SITE AND EVOLUTIONARY ORIGIN OF THE SPLICEOSOME

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The spliceosome is a dynamic molecular machine, which catalyzes the excision of introns from pre-mRNA and ligation of exons to produce continuous protein coding sequences. It comprises five RNA-protein complexes, namely U1, U2, U4, U5 and U6 small nuclear ribonucleoprotein particles (snRNPs), and various non-snRNP factors, which assemble on pre-mRNA substrates in a highly ordered manner. After the binding of U1 snRNP to the 5' splice-site (5'-SS) and U2 snRNP to the branch-point (BP) in the pre-mRNA, the remaining three snRNPs join the complex as U4/U6.U5 tri-snRNP. The spliceosome then undergoes a series of extensive structural and compositional changes to become catalytically active for the two successive trans-esterification reactions. Three U5 snRNP proteins, the DExD/H-box family helicase Brr2 (1), the EF2-like GTPase Snu114 and Prp8, play crucial roles in the activation of the spliceosome. U1 snRNP is displaced from the 5'SS by the activity of Prp28 helicase and the 5'SS intron sequence becomes paired with nucleotides within the conserved ACAGAGA sequence in U6 snRNA. The extensively base-paired U4/U6 snRNA duplex is unwound by Brr2 helicase and U6 snRNA becomes extensively base-paired with U2 snRNA so that pre-mRNA substrate, U2, U6 and U5 snRNAs form an intricate network bearing some structural resemblance to group II intron self-splicing RNA. Prp8 is the largest and most conserved protein in the spliceosome. It cross-links extensively with this catalytic core of RNA. We solved the crystal structures of yeast Prp8 (residues 885-2413) in complex with the U5 snRNP assembly factor Aar2 (2), and Brr2 with the Jab1/MPN domain of Prp8. The structure of Prp8 reveals new tightly associated domains of Prp8 resembling bacterial group II intron reverse transcriptase and type II restriction endonuclease. This architecture is very similar to group II intron encoded protein suggesting that they have a common evolutionary origin. Suppressors of splice site mutations and an intron branch-point cross-link map to a large cavity formed by the reverse transcriptase thumb, endonuclease-like and the RNaseH-like domains. The Prp8 structure has provided crucial insights into the architecture of the active site of the spliceosome and reinforced the notion that nuclear pre-mRNA splicing and group II intron splicing have a common evolutionary origin. (1) Galej, W. P., Oubridge, C., Newman, A. J. and Nagai, K. (2013). Nature 493, 638-643. (2) Nguyen, T.H.D., Li, J., Galej, W. P., Oshikane, H., Newman, A. J. & Nagai, K. (2013). Structure 21, 910-919.
INVOLVEMENT OF P73, A P53-FAMILY MEMBER, IN METABOLISM AND SENESCENCE

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p63 and p73 have been identified as the ancestral members of the p53 family. Despite the high sequence and structural similarity, the mouse knockouts revealed a crucial role in neural development for p73 and in epidermal formation for p63. We identified several transcriptional targets, the mechanisms of regulation of cell death, and the p63 isoform involved in epithelial development. We also contributed to elucidate the respective stability (JG Gong, Nature 1999) and degradation patterns via the Ub E3 ligases ITCH (M Rossi, EmboJ 2005), FBXO45 (A Peschiaroli, Oncogene 2009), antizyme pathway (I Dulloo, PNAS-USA 2010) and PIR (BS Sayan, PNAS-USA 2010). Both genes are involved in female infertility and maternal reproduction (AJ Levine, Nature Rev MCB 2011) as well as in cancer formation (G Melino, CDD 2011; P Tucci PNAS-USA 2012), although with distinct mechanisms. TAp73 knockout mice (TW Mak, G&D 2008) show high tumor incidence with hippocampal dysegensis (MAgostini, PNAS-USA 2012). Conversely, ΔNp73 knockout mice (TW Mak, G&D 2010) show a very low incidence of cancer, with sign of moderate neurodegeneration with a significant loss of cellularity in the cortex. This indicate a tumor suppressor role for TAp73 and an oncogenic role for ΔNp73. Here, we describe the involvement of p73 in senescence and metabolism. TAp73-null mice show a significant premature spontaneous aging phenotype at 14 months of age: alopecia, epidermal thinning, reduced subcutaneous fat, increased visceral fat TAp73, osteoporosis with scoliosis. This indicates that TAp73 protects against aging by regulating mitochondrial activity and preventing ROS accumulation. Indeed, both in vivo and in vivo TAp73-null mice show unbalanced mitochondrial redox defences, at least in part mediated by a direct transcriptional regulation of Cox4i1 (A Rufini, G&D 2012). TAp73 is also able to drive the expression of glutaminase type 2 (GLS2), acting on specific binding sites present on its promoter, and regulate the synthesis of serine. In agreement with these in vitro data, TAp73-null cells show clear metabolic defects in the glutamine/serine pathway affecting GSH and redox balance. In keeping, we show a role for TAp73 in the regulation of metabolic pathways.
PLE-WED-14

OF NATURE AND NURTURE: CONNECTING RNA BIOLOGY AND METABOLISM

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RNA-binding proteins (RBPs) orchestrate virtually all aspects of RNA biology. I will describe the development of “mRNA Interactome capture” as a technique that allows to chart “all” active RBPs in living cells and that led to the identification of hundreds of new RBPs from HeLa cells (1,2). The described method is broadly applicable to study mRNA interactome composition and dynamics in varied biological settings and cell types. Interactome data from murine embryonic stem cells (3), hepatocytic Huh-7 cells and yeast (unpublished) shed new light on diverse aspects of RNA biology, including RBPs in disease and novel RNA-binding architectures (4). We identify dozens of enzymes of intermediary metabolism that moonlight as RBPs in vivo, implicating these in the recently proposed REM (RNA/Enzyme/Metabolite) networks for the coordination of cell metabolism and gene expression (5).

THE JUNCTIONAL HAND-SHAKE: HOW THE PHYSICAL WORLD HELPS US UNDERSTAND THE BIOLOGY OF CELLS

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My lab seeks to understanding the cellular basis for cadherin-mediated morphogenesis. We focus on E-cadherin, the prototypical classical cadherin found in epithelial cells, which is necessary for tissue patterning and whose dysfunction contributes to cancer invasion and metastasis. An important question is what we actually mean, when we talk about “function” and “dysfunction”. Adhesion means different things to different people, from the biophysical process of resistance to detachment to the morphogenetic integrity of tissues in animals during development. These correspond to different levels of analysis, which ultimately condition how we think about function and dysfunction. The great challenge is to find ways to map between these different levels of analysis. Our approach to this problem has focused on trying to understand how cells establish cooperativity between E-cadherin adhesion and the actomyosin cytoskeleton. Increasingly, we have come to appreciate that such cooperativity reflects the interaction between intrinsically dynamic systems, which can be tuned to generate different functional outcomes. In this we have been aided by the realization that cadherin junctions are mechanically-active structures, whose ability to generate and sense mechanical force is a key to understanding their biology. In our recent efforts, we have combined quantitative dynamic live-cell imaging; tools to measure mechanical forces generated at junctions; along with mathematical modelling of active fluids in order to understand the mechanobiology of junctions. I will outline how our exploration of the physical events that occur when cells connect with their neighbours has come to influence our thinking about adhesive function and dysfunction.
STRUCTURE AND MECHANISM OF RESPIRATORY COMPLEX I, A GIANT MOLECULAR PROTON PUMP

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NADH-ubiquinone oxidoreductase (complex I) is the first and largest enzyme in the respiratory chain of mitochondria and many bacteria. Mutations in complex I lead to most common human genetic disorders. It is an L-shaped assembly, with the hydrophobic arm embedded in the membrane and the hydrophilic arm protruding into the bacterial cytoplasm or mitochondrial matrix. We have determined all currently known atomic structures of complex I. Initially, we have solved the crystal structure of the hydrophilic domain of complex I from *Thermus thermophilus*, revealing the arrangement of NADH, flavin and nine Fe-S clusters. Seven conserved clusters form a uniquely long (about 95 angstroms) electron transfer chain. Subsequent structure of the hydrophilic domain reduced by NADH revealed significant conformational changes at the interface with the membrane domain, with implications for the coupling mechanism. More recently, we have described the low-resolution architecture of the entire complex I from *T. thermophilus* and determined the crystal structure of the membrane domain of complex I from *E. coli*. It has revealed unusual novel fold of antiporter-like subunits, each containing single putative proton-translocation channel formed from two connected half-channels. The mechanism of coupling between the electron transfer in the hydrophilic domain and proton translocation in the membrane domain of complex I is not yet established, mainly because the structure of the entire complex was not known. We have now solved the atomic structure of the intact complex from *T. thermophilus*. It includes the previously unresolved key subunit ND1/Nqo8/NuoH at the interface between the two main domains and the quinone-binding site. The structure suggests that a unique, out-of-the-membrane quinone reaction chamber allows the redox energy to drive concerted long-range conformational changes in the four antiporter-like domains, resulting in translocation of four protons per cycle. Many major mechanistic implications provided by the new structure will be discussed.
LIGHT SIGNALLING NETWORKS IN PLANT DEVELOPMENT

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In addition to its role in photosynthesis, light affects plant growth and development as a signal. The light environment perceived by specific photoreceptors including phytochrome, cryptochrome, phototropin and UVR8 provides information that helps plants to adjust their body form and function to the prevailing conditions. When dark-grown seedlings become exposed to light, the photoreceptors reduce the activity of the E3 ligase CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1). In darkness, COP1 targets selected transcription factors required for photomorphogenesis to degradation. Light releases these targets from the negative regulation imposed by COP1, their pools build up allowing the progress of photomorphogenesis. In addition, upon light exposure active phytochromes migrate to the nucleus, where they bind the bHLH transcription factors PHYTOCHROME INTERACTING FACTORS (PIFs). PIFs are repressors of photomorphogenesis and phytochrome binding reduces their activity by causing their degradation in the proteasome and/or by reducing their binding to DNA targets. When light-grown plants are exposed to shade, photoreceptor activity is reduced, PIF activity is increased and the plant acquires a more competitive body form. One of the current hypotheses is that the responses to shade are also caused by increased COP1 activity. These short pathways between light signals and changes in expression of target genes describe a rather simple scenario but the key players are profusely connected to regulatory loops. This creates a rather intricate signalling network with links to the circadian clock, hormone signalling, defences, temperature signalling, etc. We are beginning to learn the functional dynamics of these multiple connections.
ENSEURING FAITHFUL SECRETION: PROTEIN QUALITY CONTROL IN THE EARLY SECRETORY PATHWAY

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Eukaryotic cells dedicate significant resources to the efficient and accurate secretion of approximately one-third of their proteome. This process begins in the endoplasmic reticulum (ER), which contains abundant chaperones that facilitate protein folding. Correctly assembled proteins are packaged into transport vesicles that deliver both protein and lipid cargoes to downstream compartments. Vesicle formation represents a key step in the quality control process: only correctly folded proteins seem to be competent for forward traffic. Yet the mechanistic basis for this quality control surveillance remains poorly understood. We have taken a “cargo-centric” approach to discover proteins that influence the biogenesis of a model misfolded protein, the yeast ABC transporter, Yor1. Broadly analogous to human CFTR, mutations in which cause cystic fibrosis, Yor1 acts at the plasma membrane as a drug pump to confer resistance to oligomycin. Misfolding mutations cause ER retention and proteasomal degradation, leading to oligomycin sensitivity. This phenotype affords a rapid and robust screen for mutations that enhance or suppress the ability of cells to tolerate increasing concentrations of drug. Using a high throughput genetic screen, we measured the effect of each non-essential yeast gene on growth conferred by Yor1-ΔF, equivalent to the predominant disease-causing allele in CFTR. We discovered that the cargo adaptor protein, Erv14, was required for forward traffic of wild-type Yor1 despite the presence of a well-conserved ER export signal on Yor1 itself. Using additional in vivo mutagenesis combined with in vitro assays, we show that the primary driver of ER export is the interaction between the Yor1 di-acidic signal and the COPII cargo adaptor, Sec24. Erv14 further enhances ER export of Yor1 through direct interaction via a site in a transmembrane domain, suggesting a membrane chaperoning function for this highly conserved ER protein.
PLE-WED-19

TUMOR-HOST DIALOGUE DURING TUMOR PROGRESSION AND THERAPEUTIC RESISTANCE

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Cancers are heterogeneous entities in which tumor cell populations as well as distinct and diverse host cell constituents form a dynamic and interactive tumor community that is pivotal not only for the genesis and progression of a tumor but also for the tumor’s ability to resist therapeutic elimination. This is in part attributed to the inherited and acquired skills of malignant cells to manipulate and alter the environment rendering it more submissive to the tumor’s needs. The tumor vasculature entails an integral and critical component that besides its expanding ability to fuel tumors sufficiently with oxygen and nutrients serves the purpose of providing a protective niche for tumor stem cells thereby regulating their maintenance and propagation. In addition, blood vessels are a multifaceted traffic system enabling tumor cells to travel to distant organs, and bone marrow-born cells to infiltrate the tumor. Much less studied, but equally important, is their use as tracks aiding tumor cells to invade into normal neighboring tissue by migrating along the outer surface of blood vessels. An overview will be presented of the crosstalk between the tumor and host cells during tumor progression and targeted therapies.
PLE-TUH-20

A ROADMAP FOR TRANSLATIONAL NANOMATERIALS AND TECHNOLOGIES AIMED AT RESTORING NEUROLOGICAL FUNCTION

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Nanoengineering and nanotechnology offer unique opportunities to interface with neurons both for stimulation and recording. The design, control, and properties of these technologies often result in effects at the molecular and cellular scales that are the direct result of the fabrication and engineering of such technologies at the nanoscale. Emerging work by our group and others are beginning to show that nanotechnologies designed to electrically interface with neurons can result in signaling effects and phenomena not seen with other technologies at other scales, e.g. microtechnologies and electrodes. Related work has focused on the development of nanomaterials and nanoparticles for the regeneration of the central nervous system, imaging of structure (anatomy) and function, and for drug delivery. While the potential of these technologies are significant, there remain many open questions and challenging engineering and neurobiological issues to be defined and resolved. It remains to be determined what the eventually impact such technologies may have in the lab and eventually the clinic. This lecture will begin by providing an overview of the literature to date, then attempt to define some of the types of open problems and questions that need to be addressed. We will end by speculating on where this exciting area of neural engineering and neurobiology research needs to go, and what some of the potential outcomes might be. We will also highlight some of our own experiences in attempting to accelerate and push the research envelope through industry-academic partnerships, and what the pros and cons of doing so are.
CHROMATIN MODIFICATIONS IN PLANT STRESS RESPONSES AND STRESS MEMORY

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As sessile organisms plants have to adapt to fluctuating environmental conditions including abiotic stress factors such as salinity and drought. The adaptive process occurs at different time scales ranging from minutes to thousands of years. There is increasing evidence that epigenetic, chromatin-based, processes contribute to stress adaptation. For example, changes in the histone acetylation status of dehydration-responsive genes regulate the transcriptional response to acute water stress, while stress-induced changes in DNA methylation can re-activate transposons and lead to enhanced mutation frequency and hence to increased adaptive potential in subsequent generations. Recent research in our laboratory has generated new knowledge on the function of histone modifications in both short-term and long-term responses of Arabidopsis thaliana plants to salt and drought stress:

1. **Function of histone deacetylation in acute stress responses:** Searching for putative components of plant histone deacetylase complexes, we identified a gene with partial homology to a hitherto uncharacterized member of the yeast Rpd3 complex. Histone Deacetylation Complex (HDC) 1 is encoded by a single-copy gene in most plant genomes. In A. thaliana, HDC1 is a ubiquitously expressed nuclear protein that interacts with at least two deacetylases (HDA6 and 19), promotes histone deacetylation, and attenuates de-repression of genes under salt stress. The fast-growing HDC1-overexpressing plants outperformed wildtype plants not only on well-watered soil but also when water supply was reduced. Our findings identify HDC1 as a rate-limiting component of the histone deacetylation machinery and as an attractive tool for increasing biomass and water usage efficiency in plants.

2. **Function of histone demethylation in somatic stress memory:** Chromatin marks provide a potential molecular mechanism for somatic plant memory and priming. To investigate whether transient exposure of seedlings to salt stress causes chromatin changes and whether these are maintained throughout vegetative growth we established an effective protocol for salt priming in the model plant Arabidopsis. Primed plants were identical to non-primed plants in growth and development, yet they displayed reduced salt uptake and enhanced drought tolerance during a second stress exposure. ChIP-seq analysis of four histone modifications revealed that the priming treatment altered the epigenomic landscape; changes were small but tissue-specific, varied in number and direction depending on the modification, and preferentially targeted transcription factors. Notably, priming led to shortening and fractionation of H3K27me3 islands (‘etching’). This effect faded over time, but was still apparent after a ten day growth period in control conditions. Several genes with priming-induced differences in H3K27me3 showed altered transcriptional responsiveness to the second stress treatment.

PLE-THU-22

A QUARTER CENTURY OF HEDGEHOG SIGNALLING

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The Hedgehog signaling pathway was initially elucidated through the analysis of the \textit{Drosophila} segment polarity genes, first described by Nüsslein-Volhard and Wieschaus in their seminal \textit{Nature} paper published in 1980. The cloning of vertebrate homologues of \textit{Drosophila hedgehog} exactly 20 years ago led to the elucidation of the key role played by \textit{Sonic Hedgehog (Shh)} in patterning the vertebrate embryo. In addition, dysfunction of the Hedgehog signaling pathway has been implicated in a variety of cancers as well as congenital disorders, stimulating a concerted effort to discover drugs that modulate the pathway. Analysis of the mechanisms and functions of Shh in the zebrafish have revealed both conserved and divergent aspects of Hedgehog signalling between species. I will review some of these findings and also present the results of our recent studies of the kinesin-family member Kif7, the vertebrate orthologue of \textit{Drosophila Costal2}, mutations of which are associated with a number of human pathological disorders including Acrocollosal and Joubert syndromes. Our findings highlight the complexity of the Hedgehog signal transduction pathway and underline the value of a multi-systems approach to its analysis.
MEASURING PLANT GROWTH

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Plants pace their metabolism and growth in the face of a fluctuating environment. The daily alternation between light and darkness has an especially marked impact on plants, because photosynthesis is only possible in the light. Metabolism and growth in the night depend entirely on reserves, like starch, that are accumulated in the light and remobilised in the dark. Starch turnover is exquisitely regulated by the clock and metabolic signals, pacing starch breakdown such that the reserves are almost but not entirely consumed at dawn. This must be accompanied by coordinated by changes in the growth rate. Growth has been intensively researched in plants, but usually by monitoring physical size, e.g., leaf expansion. As mature plants cells are highly vacuolated, expansion is mainly due to water uptake. I will discuss approaches that give information about the synthesis of cellular components. One approach is based on whole plant carbon balances, linked with modelling to obtain time-resolved estimates of growth. Another uses quantitative data on ribosome abundance, polysome loading and transcript abundance to model the rates of protein synthesis and, additionally, provide insights into protein turnover and the associated costs. The third involves supplying $^{13}$C-$\text{CO}_2$ to intact plants and analysing the labelling kinetics of metabolic pools and cellular components like proteins. I will discuss (i) how growth is distributed in a highly flexible manner between the day and the night to optimising ribosome use to the prevailing conditions, (ii) how changes in ribosome abundance and usage and protein turnover contribute to differences in growth rates in wild Arabidopsis accessions and (iii) how ribosome abundance plays a key role in growth strategies that allow native Banksia and Hakea species to grow on the extremely phosphate-deficient soils in south-western Australia.