

PLENARY LECTURES

Monday - Friday

PLE-MON-01**IMMUNITY TO INFLUENZA VIRUSES**

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The presentation will consider the fundamentals of the influenza problem and where we are with the H5N1 "bird" flu epizootic and H1N1 "swine" flu pandemic. How big a threat is influenza to human and animal populations? What does history tell us about this disease? The nature of immunity to the influenza A viruses will be discussed, together with possible novel vaccination strategies. The basic characteristics of the virus-specific CD8+ "killer" T cell response will then be considered, with particular emphasis on both immune recognition and the molecular events that lead to the emergence and maintenance of differentiated phenotypes. Immune T lymphocytes offer a readily manipulated, and well-defined system for understanding the basics of epigenetic control. Considerable progress is being made in understanding the similarities and differences between naïve, effector and memory CD8+ T cells.

PLE-MON-02**REGULATORY NETWORKS IN THE DEVELOPING HEART****Harvey R.P.**

Victor Chang Cardiac Research Institute, Level 7, 405 Liverpool, Darlinghurst, NSW 2010, Australia.

Professor Richard Harvey received his PhD in 1982 from the Department of Biochemistry, University of Adelaide, after having trained in molecular biology under Julian Wells. Following a brief sojourn in biotechnology in Strasbourg, France, he undertook postdoctoral studies over 3 years with Doug Melton at Harvard University, adapting molecular techniques to the analysis of embryonic development. He then moved to the Walter and Eliza Hall Institute of Medical Research in Melbourne where he established an independent research effort. In 1998, after 10 years at WEHI, he moved to the Victor Chang Cardiac Research Institute, where he is currently Co-Deputy Director and Head of the Developmental Biology Program, and holds the endowed Sir Peter Finley Professorship of Heart Research at the University of New South Wales. He is an Associate Member of EMBO and in 2007 was elected member of the Australian Academy of Science. He currently holds an NHMRC Australia Fellowship and is Leader of the Adult Stem Cells Collaborative Stream of the Australian Stem Cell Centre. Since the early 1990s, Richard's research has focused on the genetic basis of heart development and congenital heart disease, largely using the mouse as a genetic model but also applying key insights to human populations. In the early 1990s, he described the homeodomain transcription factor Nkx2-5, which provided a key entry point for genetic dissection of heart development and disease. His subsequent studies on Nkx2-5 and other core cardiac transcription factors have help move cardiac development and congenital heart disease research into the molecular era, and have contributed important insights into cardiac evolution, the nature of cardiac patterning, and the cellular and molecular basis of congenital heart disease pathology. More recently he has begun an exploration of the biology and origin of adult cardiac stem cells, and cardiac regeneration. Progressively, his work shifts towards a more systems level understanding and the challenges of network biology will occupy much of his attention in the coming years.

PLE-MON-03

EARLY ATMOSPHERIC CO₂ SENSING AND DROUGHT-INDUCED ABSCISIC ACID SIGNALING MECHANISMS IN PLANTS

Schroeder J.I.¹, Nishimura N.¹, Hu H.¹, Xue S.¹, Murata Y.², Park S.Y.³, Cutler S.³, Hitomi K.⁴, Arvai A.⁴ and Getzoff E.⁴

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The continuing rise in atmospheric CO₂ causes closing of stomatal pores and regulates stomatal development and thus globally affects CO₂ influx into plants and plant water transpiration. Under drought stress conditions, the plant hormone abscisic acid triggers stomatal closing, thus inhibiting plant water loss. Stomatal pores are formed by guard cell pairs that control stomatal opening and closing. Guard cells have been developed as a model system for dissecting ion channel functions and time-resolved early signal transduction mechanisms in plant cells. Elevations in atmospheric CO₂ and the hormone abscisic acid (ABA) both trigger signaling cascades in guard cells that result in stomatal closing and in reducing plant water loss. However, the CO₂ perception mechanisms had remained unknown and a new ABA receptor class has recently been identified (Ma et al., 2009 *Science*; Park et al., 2009 *Science*). New findings will be presented for CO₂ binding proteins that are essential for triggering early CO₂ signal transduction in guard cells (Hu et al., 2010 *Nature Cell Biol.*). Furthermore, new genetic loci and mechanisms that mediate CO₂ control of plant gas exchange will be presented. The PYR/RCAR proteins were recently identified as ABA receptors in plants (Park et al., *Science* 2009; Ma et al., *Science* 2009). Independent proteomic identification of these proteins as major ABI1 phosphatase-interacting proteins *in planta* and their functions in guard cell signaling will be presented (Nishimura et al., *Pl. J* 2010). Furthermore, advances will be presented at identifying the structure-based mechanisms by which ABA stimulates PYR/PYL/RCAR signaling (Nishimura, Hitomi, Arvai et al., *Science* 2009). Many studies have shown a central role for intracellular Ca²⁺ in guard cell ion channel regulation leading to stomatal closing. The mechanisms mediating Ca²⁺ specificity in signaling are subjects of present investigation in animal and plant systems. Signal transduction analyses will be presented, that point to a mechanism for how guard cells can achieve specificity in CO₂ and ABA-induced calcium signaling through enhancing (priming) the sensitivity of intracellular Ca²⁺ signaling mechanisms.

PLE-TUE-04**MECHANISMS OF miRNA-MEDIATED GENE SILENCING****Izaurralde E.**

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MicroRNAs (miRNAs) are genome-encoded ~22 nucleotide-long RNAs that silence gene expression post-transcriptionally by base pairing with the 3' untranslated regions of target mRNAs. To exert their function, miRNAs associate with Argonaute proteins (AGOs) in miRNA-induced silencing complexes (miRISCs), which silence the expression of mRNAs containing partially or fully complementary miRNA-binding sites. In animals, most miRNAs are only partially complementary to their targets. In this case, our group has shown that the AGO proteins are not sufficient to mediate silencing and require interaction with proteins of the GW182 family. We have also shown that AGO-GW182 complexes mediate silencing by promoting translational repression and mRNA deadenylation catalyzed by CAF1-CCR4-NOT, the major cytoplasmic deadenylase complex. Deadenylation decreases translation efficiency and, in somatic cells, commits the mRNA to decapping and 5'-to-3' exonucleolytic degradation. Our analysis of GW182 protein function has revealed two domains critical for silencing: an N-terminal GW-repeat-containing region conferring binding to AGOs, and a bipartite silencing domain, consisting of Mid and C-terminal regions, which elicits translational repression and degradation of miRNA targets. Exactly how the bipartite silencing domain of GW182 proteins interferes with translation and accelerates deadenylation is not completely understood. We have recently started to address this question by showing that the silencing domains of GW182 interact with the cytoplasmic poly(A)-binding protein 1 (PABPC1), suggesting GW182 proteins are PABP-interacting proteins (Paips) that interfere with the function of PABPC1 in translation and mRNA stabilization.

PLE-TUE-05

PHYSIOLOGICAL ROLE OF AUTOPHAGY AND ITS REGULATION MECHANISM**Mizushima N.**

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Autophagy is the primary pathway for the degradation of cytoplasmic constituents in the lysosome. When autophagy is induced, a portion of cytoplasm is sequestered by autophagosomes, and then delivered to lysosomes. Yeast genetic studies have identified more than 30 *ATG* genes, which are required for autophagosome formation. These *ATG* genes are highly conserved in higher eukaryotes including mammals. Based on these findings, we have analyzed physiological roles of autophagy using several mouse models. Using autophagosome-indicator mice (GFP-LC3 transgenic mice) and conventional *Atg5* knockout mice, we have shown that autophagy is up-regulated during starvation and is critically important to tide over starvation such as during the early neonatal period. We also discovered that Autophagy is essential for preimplantation development of mouse embryos. The level of autophagy is low in unfertilized oocytes; however, autophagy is upregulated shortly after fertilization. Analysis of oocyte-specific *Atg5* knockout mice revealed that autophagy-defective embryos failed to develop beyond the 4- and 8-cell stages. We suggest that degradation of maternal proteins by autophagy is critical to produce necessary amino acids during preimplantation development in mammals. Thus, "induced autophagy" is critical to produce necessary amino acids during starvation and preimplantation development. On the other hand, we found that constitutive "basal" autophagy is important for intracellular protein quality control, because multiple protein aggregates accumulate in the cytoplasm of neural cell- and liver-specific *Atg5* knockout mice. Finally, to further analyze the effects of defects in autophagy for a long period in vivo, we generated mice with mosaic deletion of *Atg5*. These mice are viable for more than 19 months and do not show obvious phenotype during the first several months. However, after 9 months of age, multiple tumors develop in the liver, which are derived from *Atg5*KO cells. Tumors were not detected in other organs. These results suggest that autophagy is important for suppression of spontaneous tumorigenesis in the liver. We have also conducted studies on molecular mechanism of autophagy regulation and autophagosome formation. We recently identified several new mammalian autophagy factors such as FIP200, mAtg13, Atg101 and mAtg14. We thus determined hierarchical relationships among these mammalian autophagy factors and found that the ULK1-mAtg13-FIP200-Atg101 complex is the most upstream unit and is directly regulated by mTORC1. Upon starvation, the ULK1 complex localizes to punctate structures associated with the ER. We hypothesize that these structures represent autophagosome formation sites in mammalian cells.

PLE-TUE-06

MECHANISMS OF PROTEIN TRANSPORT ACROSS MEMBRANES**Rapoport T.A.**^{1,2}¹Harvard Medical School. ²Howard Hughes Medical Institute.

Many proteins in bacteria are transported during their biosynthesis across or are integrated into the plasma membrane, a process that is similar to protein translocation across endoplasmic reticulum (ER) membrane in eukaryotes. Transport occurs through a protein-conducting channel that is formed from a conserved heterotrimeric membrane protein complex (SecY or Sec61 complex). The channel associates with different partners in different translocation pathways. In bacteria, the SecY channel can associate with the translating ribosome (co-translational translocation) or with the cytoplasmic ATPase SecA (post-translational translocation). The crystal structure of an archaeal SecY complex shows the architecture of the closed channel. The structure suggests that a single SecY copy forms the translocation pore, a prediction that was confirmed for SecA-mediated translocation by disulfide bridge crosslinking experiments. However, translocation appears to be mediated by oligomers of the SecY complex; nucleotide-binding domain 1 (NBD1) of SecA interacts with a non-translocating SecY copy, while other domains of SecA “push” the polypeptide chain through a neighboring SecY copy. A crystal structure of a SecA-SecY complex shows one copy of SecA in its transition state of ATP hydrolysis bound to one copy of SecY complex. The latter corresponds to the translocating SecY copy. Both SecA and SecY undergo major conformational changes upon interaction. The structure indicates that SecA binding opens the lateral gate of SecY for signal sequence intercalation, and induces plug movement. In addition, the data suggest how the polypeptide chain moves through SecA and SecY, a path confirmed by disulfide bridge crosslinking experiments. The polypeptide moves through a “clamp” of SecA and passes by the tip of the “two-helix finger” before entering the SecY pore. We propose that upon ATP binding to SecA, the two-helix finger moves towards the SecY pore, using a crucial tyrosine residue at the fingertip to drag the polypeptide chain with it. Simultaneously, the clamp would loosen its grip on the polypeptide. Following ATP hydrolysis, the clamp would tighten and the finger would reset to grab the next polypeptide segment. These events would be repeated until the polypeptide is fully translocated through the channel.

PLE-TUE-07

FLYING IN THE FACE OF NEURODEGENERATION WITH DROSOPHILA**Bonini N.M.**^{1,2}¹University of Pennsylvania. ²Howard Hughes Medical Institute.

Human neurodegenerative diseases, like Huntington's disease and the spinocerebellar ataxias, are late-onset progressive neurodegenerative disorders for which few cures or treatments are available. To develop new approaches, we are using the simple model organism *Drosophila* as a model. We use the human disease gene to recreate the disease in the fly, and then take advantage of powerful molecular genetic approaches in the fly to define modifiers and mechanisms. These studies have revealed that molecular chaperones dramatically modulate disease phenotypes. They have also revealed a pathogenic role of the CAG repeat RNA encoding the disease protein in the polyglutamine disease SCA3. Our studies of applying genetics to these situations are therefore revealing new approaches to modulate disease progression, and are highlighting new common components of RNA-based and polyQ-protein based repeat expansion diseases. Our particular focus recently has been on the role of RNA-mediated pathways in disease, and on the role of RNA binding proteins to cause disease. We are developing new models, as well as integrating modulatory pathways between different disease models, to provide insight into common mechanisms and pathways affected across distinct human neurodegenerative diseases.

Sponsored by Sigma Aldrich Pty Ltd

PLE-WED-08

EPIGENETICS AND THE DETERMINATION OF PHENOTYPE

Whitelaw E.

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It is well recognised that there is a surprising degree of phenotypic variation among genetically identical individuals even when the environmental influences, in the strict sense of the word, are controlled. Genetic textbooks acknowledge this fact and refer to it as “intangible variation” or “developmental noise”. We suggest that this intangible variation results from the stochastic establishment of epigenetic modifications to the DNA nucleotide sequence in early development. These modifications, which involve DNA methylation and chromatin remodelling, result in alterations in gene expression, which, in turn, affect the phenotype of the organism. Work from a number of labs suggests that the establishment of these epigenetic states can be influenced by the environment raising the possibility that they are involved in the developmental origins of some disease states. We have recently shown that the consumption of 10% ethanol during the first half of gestation in the mouse alters the DNA methylation levels at a gene that is particularly sensitive to epigenetic state. We have carried out a “sensitized” ENU mutagenesis screen in the mouse to identify genes that modify epigenetic state. In most cases they are homozygous lethal, indicating the obligate requirement for the genes that have been hit (Blewitt et al, 2005). So far, we have identified ten of the underlying mutations, some of these are novel. Most tested so far affect expression at epigenetically sensitive loci such as the agouti viable yellow allele. We have now screened 4000 F1s and identified 40 MommeDs. This project has the potential to identify many more novel genes involved in epigenetic phenomena, and to produce hypomorphs and hypermorphs of known modifiers of epigenetic state. Mice haploinsufficient for such proteins show a range of subtle phenotypes, including obesity and behavioural abnormalities. These mutant lines will be a valuable resource to study the role of epigenetics in gene / environment interactions. Funding from the National Health and Medical Research Council of Australia and the Australian Research Council.

Sponsored by Functional Plant Biology

PLE-WED-09

EPIGENETIC REGULATION OF CAROTENOID COMPOSITION AND PLANT DEVELOPMENT IN RESPONSE TO A CHANGING ENVIRONMENT

Cazzonelli C.I., Burgess A.L., Roberts A.C., Yadav A.K., Collinge D. and Pogson B.J. Centre of Excellence in Plant Energy Biology, Research School of Biology, The Australian National University, Building 41 Linnaeus Way, Canberra, ACT, Australia 0200.

Carotenoids are natural pigments comprising the breathtaking yellow to red colours of many fruits, vegetables, flowers, butterflies, birds and fish, as well as they promote health and are used as behavioural cues in animals. In plants, carotenoids are required for photosynthesis, photoprotection and the biosynthesis of at least two hormones, namely abscisic acid and strigolactones. Carotenoid composition is finely tuned in plant tissues to enable cellular adaptation in response to developmental and environmental cues. The carotenoid biosynthetic pathway bifurcates after lycopene to produce lutein or beta-carotenes and its derivatives. Thus the branch point modulates which carotenoids accumulate (Cazzonelli et al., 2010 Trends in Plant Sciences). We have shown how the branch point can be regulated by a chromatin-modifying histone methyltransferase, Set Domain Group 8, (SDG8), targeting the carotenoid isomerase (*CRTISO*) (Cazzonelli et al., 2009 Plant Cell). SDG8 controls the permissive expression of a small number of genes by histone methylation of lysine 4 and/or 36 of chromatin surrounding key gene targets such as *CRTISO* (Cazzonelli et al., 2009 Plant Signaling and Behavior). Regions within the *CRTISO* promoter are required for SDG8 recruitment as well as function, and tissue specific expression of *CRTISO* is similar to that of *SDG8* (Cazzonelli et al., 2010 Molecular Plant). Interestingly, *sdg8* and *crtiso* mutants display similar phenotypes such as altered root architecture, enhanced shoot branching, male sterility and reduced seed set, depending on the environmental conditions. We are exploring the molecular nature by which SDG8 regulates *CRTISO* and how modulating carotenoid flux through the pathway may perturb the production of carotenoid-derived signaling molecules. The chromatin modifying nature of SDG8 and novel functions for *CRTISO* in regulating plant development in response to environmental change have opened a new door to improve our understanding of epigenetic processes.

PLE-WED-10**THE EVOLUTION OF THERAPEUTIC MONOCLONAL ANTIBODIES****Winter G.**

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Over recent years we have seen a range of therapeutic antibodies bursting onto the market for treatment of cancer and immune disorders. The success of antibodies has been dramatic; antibodies are displacing small molecule drugs in the league tables of top-selling drugs, and by 2014 it is estimated that the three top selling drugs will be antibodies. Almost all of the marketed therapeutic antibodies are full IgG molecules; most are genetically engineered from rodent hybridomas but more recently we have seen genetically engineered human antibodies made from phage display technology or from “human” mice appear on the market. We have seen efforts to shrink antibodies, including use of single antibody domains for antigen binding, and drug conjugates for cell killing; we have also seen efforts to build antibodies with additional antigen-binding activities. Perhaps the ultimate in shrinking antibodies is the development of bicyclic peptides attached to a chemical core. These “bicycles” have the potential to mimic antibodies, but are close to the size of conventional small molecule drugs, and may thereby combine the advantages of biologics and chemical drugs. I will outline the evolution of technologies and strategies underpinning the evolution of antibodies to the present, and possible futures.

PLE-WED-11**DUPLICATION, DISEASE AND THE EVOLUTION OF THE HUMAN GENOME****Eichler E.E.**

Department of Genome Sciences, University of Washington.

Duplicated sequences are important sources for the evolution of new gene function within species. By dint of their homology, duplicated sequences are also prone to much more rapid rates of mutation due to their propensity to promote rearrangement. Hominids have a preponderance of intrachromosomal duplications organized in an interspersed fashion as opposed to tandem, which is the archetype in most other mammalian genomes. We have developed an approach to reconstruct the evolutionary history of these regions using graph theory, phylogenetics and outgroup genome sequence data. All of these data point to a burst of segmental duplications in the common ancestor of human and apes. I will show that much of the interspersed human duplication architecture is focused around core duplicons corresponding to the expansion of gene families that show strong signatures of positive selection and which lack orthologs in other mammalian species. This architecture predisposes apes and humans to extensive large-scale genetic diversity mediated by non-allelic homologous recombination. I will show that the recent evolution of this architecture has led to a high background rate of copy-number variation mutations associated with neuropsychiatric and neurodevelopmental disease in the human species.

PLE-WED-12

INVESTIGATING THE ROLE OF EXOSOMES IN THE PROCESSING OF PROTEINS ASSOCIATED WITH NEURODEGENERATIVE DISEASES**Hill A.F.**^{1, 2, 3}

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Prions are the infectious particle responsible for the transmissible neurodegenerative diseases such as Creutzfeldt-Jakob disease (CJD) of humans or bovine spongiform encephalopathy (BSE) of cattle. The principal component of infectious prions is a misfolded form (PrP^{Sc}) of the host-encoded cellular prion protein (PrP^C). The interaction between PrP^C and PrP^{Sc} is an early event in the propagation of further PrP^{Sc} molecules. We have investigated the generation of infectious prions in cell and animal models to characterise the forms of prion protein packaged into small extracellular vesicles known as exosomes. We find that exosomes from these infectious cells are capable of transmitting infection to further uninfected cells suggesting cellular co-factors necessary for the generation of prion infectivity are contained within these vesicles. Furthermore, the prion protein contained within the exosomes has distinct biochemical properties to that found in whole cells suggesting processing of this protein into these vesicles occurs through a distinct pathway. This work is mirrored by our studies on another protein involved in neurodegenerative disease, the amyloid precursor protein (APP) which is associated with Alzheimer's disease (AD). Exosomes are also involved in the processing of APP and these vesicles contain several distinct proteolytically cleaved fragments of APP as well as A β . In addition, these fragments can be modulated using inhibitors of the proteases involved in APP cleavage. We identify several key members of the secretase family of proteases to be localized in exosomes suggesting they may be a previously unidentified site of APP cleavage. These results provide further evidence for a novel pathway in which PrP and APP fragments are released from cells and has implications for the analysis of APP processing and diagnostics for prion and Alzheimer's disease.

PLE-WED-13**MEMBRANE PHYSIOLOGY IN LEAVES, EARTH'S LUNGS****Evans J.R.**

Plant Sciences, RSBS, Australian National University, ACT 0200.

Two aspects of membranes in relation to photosynthesis will be considered: their permeability to carbon dioxide and the protein complexes they contain. Leaves acquire carbon dioxide from the atmosphere for fixation by photosynthesis in exchange for water and oxygen. Certain characteristics of leaves resemble those of lungs as the morphology of both organs reflects solutions to the problem of exchanging gases. The flux in leaves is the same as that in the lungs of mammals when expressed per unit area at the gas/liquid interface. To enable greater fluxes for a given tissue volume, the surface area of mesophyll cells and alveoli are greatly ramified. However, the concentration gradients driving carbon dioxide exchange differ greatly between leaves and lungs. Consequently, the permeability of plant membranes to carbon dioxide must be orders of magnitude greater than the permeability of membranes in lungs. The prospect of being able to manipulate membrane permeability has been encouraged by the discovery that aquaporins can alter membrane permeability to carbon dioxide in red blood cells and leaf mesophyll cells. The second aspect of membrane physiology concerns thylakoid membranes within chloroplasts. These membranes are fundamental to photosynthesis as they contain the protein complexes associated with light capture, electron transport and ATP synthesis. Maximum rates of photosynthetic oxygen evolution are linearly related to the ATPase and cytochrome f contents. Opportunities to enhance photosynthesis will be raised.

PLE-THU-14**PLANT INNATE IMMUNITY PROTEINS****Anderson M.A.**

Department of Biochemistry, La Trobe University, Melbourne, Australia, and Hexima Ltd., Melbourne, Australia.

Plants lack an adaptive immunity system of the sophistication found in higher organisms. Nevertheless they have developed complex strategies to minimise infection by microorganisms and damage by insect pests. We have been working on three groups of plant innate immunity proteins. All are disulphide rich proteins and potent insecticidal or antifungal molecules. We have focused on structure, mechanism of action and ultimately on their commercial application in plant protection. Two of these groups of molecules were discovered in the flowers of solanaceous plants where they act to protect the reproductive structures and ensure seed set. The first group are serine proteinase inhibitors that target trypsin and chymotrypsin, the major digestive proteinases in the gut of Lepidopteran larvae. The way larvae respond to and survive the ingestion of these proteinase inhibitors (PIs) has been investigated and this knowledge has been applied to enhance the resistance of transgenic plants to insect attack in the field. The second group of floral defence molecules are the defensins which are potent antifungal molecules. These defensins interact specifically with the cell wall of filamentous fungi, penetrate the plasma membrane and enter the cytoplasm of the hyphae where they induce cell death. They also have application in plant protection and have been used to enhance disease resistance in transgenic cotton in the field. The cyclotides comprise the newest group of plant innate immunity proteins. These 29 amino acid cyclic peptides are common in the Rubiaceae and the Violaceae. Although new to plant science they are the most abundant cyclic proteins on earth. Their function has not been fully explored but the best characterised are potent insecticidal and nematocidal molecules. Our research focuses on their biosynthesis and mechanism of insecticidal activity.

PLE-THU-15

REGULATION BY UBIQUITIN AND UBIQUITIN-LIKE PROTEINS**Schulman B.A.**^{1,2}¹Howard Hughes Medical Institute. ²St. Jude Children's Research Hospital, Memphis, TN 38105 USA.

Post-translational covalent attachment of ubiquitin-like proteins (Ubls) to protein targets is a predominant eukaryotic regulatory mechanism. In higher eukaryotes, more than a dozen Ubls - including ubiquitin, NEDD8, ISG15, and SUMO - covalently modify myriad substrates. The best understood function of a Ubl modification is ubiquitin-mediated proteasomal degradation. However, different Ubls alter the functions of their targets in different ways, such as by changing the target's subcellular localization, enzymatic activity, or interactions with other proteins or DNA. Our goals are to understand (1) the basic enzymatic mechanisms underlying Ubl attachment to targets, (2) how Ubls are attached selectively, and (3) how Ubl modifications alter target functions. Ubls are attached to protein targets by the sequential action of enzymes in three classes, known as E1, E2, and E3. During this process, a Ubl becomes transiently covalently linked to enzymes, and ultimately to the target. This is a highly dynamic process, in which a Ubl is "handed off" first between enzymes, and ultimately to a target. I will present an overview of our recent work providing structural glimpses into the molecular principles underlying dynamic protein regulation by ubiquitin and other Ubl transfer cascades.

PLE-THU-16

STRUCTURE AND FUNCTION OF PROTEINS RELEVANT IN DRUG DISCOVERY**Wang H.-J.**

Institute of Biological Chemistry, Academia Sinica, Taipei 115, Taiwan.

Protein crystallography is a powerful tool for drug discovery. Many important drug targets can be analyzed with relevant ligands (substrates/inhibitors) bound at the active site. In the lecture, I will discuss the following enzymes and protein regulators that are useful for antimicrobial and anti-inflammatory drug development. Prenyltransferases are involved in many biological pathways; thus they are useful for developing new drugs for various diseases. We have studied several *trans*-type prenyltransferases, such as geranylgeranyl pyrophosphate synthase (GGPPS), complexed with several bisphosphonate inhibitors. In addition, dehydrosqualene synthase (CrtM) from *Staphylococcus aureus* uses the head-to-head condensation of two farnesyl pyrophosphate (FPP) molecules to produce the C30 presqualene pyrophosphate, the precursor of the golden carotenoid pigment staphyloxanthin, which promotes bacterial resistance to reactive oxygen species and host neutrophil-based killing. CrtM, therefore, has been tested as the target to treat infections by methicillin-resistant *S. aureus* (MRSA). We found squalene synthase inhibitors for cholesterol-lowering activity in humans bind to CrtM and block the biosynthesis of staphyloxanthin *in vitro*, resulting in colorless bacteria with increased susceptibility to killing by human blood and to innate immune clearance in a mouse infection model. Another study related to MRSA is on TcaR and IcaR, a weak and a strong negative regulator of transcription of the *ica* locus, respectively. Their presence prevents the poly-N-acetylglucosamine production and biofilm formation in *S. aureus* and *S. epidermidis*. We solved the 3D structure of TcaR in its apo form and in complex with salicylate as well as several aminoglycoside and β -lactam antibiotics. A comparison of the native and complex TcaR structures indicates that the regulation mechanism involves a large conformational change in the DNA-binding lobe. The antimicrobial compounds we tested were shown not only to inhibit TcaR-DNA interaction but also to further induce biofilm formation in *S. epidermidis* in our *in vivo* assay. The results support a general mechanism for antibiotics in regulating TcaR-DNA interaction and thereby help understand the process of bacterial antibiotic resistance through biofilm formation. Finally the role of glutaminy cyclase, which makes N-terminal pyroglutamate, in the inflammatory process will be addressed.

PLE-THU-17

LESSONS FROM DEATH SIGNALING**Dixit V.**

Genentech, Inc. 1 DNA Way South San Francisco, CA 94080.

Antimitotic drugs are widely prescribed and highly effective chemotherapeutics; but, the precise mechanism by which they promote apoptosis is unknown. We find that Mcl-1, a member of the Bcl-2 family of anti-apoptotic proteins is a critical modulator of this cell death process. During mitotic arrest, levels of this highly labile, but potent pro-survival factor, dramatically decline sensitizing cells to apoptosis. The fall in Mcl-1 protein is mediated by a post-translational mechanism: the tumor suppressor, SCF^{FBW7} targets Mcl-1 for proteosomal degradation. Genetic deletion of FBW7 or inactivating mutations present in patient-derived samples block Mcl-1 degradation, enhance cell survival and attenuate cell death induced by anti-mitotic agents. Thus, for apoptosis to initiate during mitotic arrest the Mcl-1 pro-survival checkpoint must be neutralized. Patients with tumors harboring deletion or inactivating mutations in FBW7 are unlikely to benefit from anti-mitotic therapies: a consequence of persistent high levels of Mcl-1.

PLE-THU-18**STRUCTURAL BIOLOGY: CHALLENGES AND PROSPECTS****Deisenhofer J.**

The University of Texas Southwestern Medical Center, Dallas, Texas, USA.

Since the 1950s, structural biology has experienced enormous progress and has contributed to almost all aspects of biological research. Despite countless successes, challenges remain. Among them are a continuing need to push the boundaries of current techniques for structure determination of proteins and nucleic acids, as well as larger assemblies. Equally necessary are improvements of our understanding of macromolecular structures, so that we can make reliable predictions, for example, about the folding, stability, and function of proteins. Using examples from our own work, I will discuss open questions in modeling macromolecular structures and dynamics. I will also comment on recent technological developments, such as, for example, the free electron lasers coming online in Europe, the US, and Japan.

PLE-THU-19**SEEKING NEURAL TUBE CLOSURE AND PEERING INTO GENE-ENVIRONMENT INTERACTIONS**

Niswander L., Marean A., Pyrgaki C., Massarwa R. and Zhang Y.
HHMI and University of Colorado, Aurora, CO, US 80108.

The neural tube is the embryonic precursor of the central nervous system (CNS), the brain and spinal cord. The CNS tissue starts as a flat “plate” which then rolls up to form the neural tube. During this process of neurulation, the neural cells are dividing, undergoing complex patterning to form the appropriate neuronal precursors, changing their shape, and interacting with new neighbors. Failure to close the neural tube results in neural tube defects, like spina bifida and exencephaly, the second most common human birth defect. However, little is known of the genes that control neural tube closure or how these genes act. To gain insight into this complex morphogenetic process, we have used an unbiased approach of forward genetic screening in mice to identify a number of genes that are critically required for neural tube closure. Our goal is to clone the genes which when mutated cause neural tube defects and to determine the mechanisms by which they act to regulate this critical embryonic process. To date we have cloned 12 new genes necessary for neural tube closure. This has led to novel insights into Hedgehog signaling, regulated proliferation, interactions between head mesenchyme and neural tissue, and neural fold closure. The mouse embryo provides a genetically tractable model of human neurulation. However, our understanding of the dynamic nature of neurulation has been hampered in the mammalian embryo due to its in utero development and therefore its inaccessibility. Using an ex utero embryo culture system coupled with live confocal microscopy imaging, we have visualized the dynamic cell behaviors comprising mouse neural tube closure. This has revealed unanticipated interactions between the cells of the juxtaposed neural folds. Our unexpected findings reveal a unique mechanism of closure in the midbrain that is distinct from the long-hypothesized zipper-like process that occurs generally throughout the hindbrain and caudal neural tube. We observe the presence numerous intermediate closure points that are preceded by long cellular extensions across the gap between the closing neural folds in the midbrain. Our next goal is to determine how cell behavior is disrupted in the various neural tube mutants. Our third and long-term goal is to understand how maternal dietary choice, in this case folic acid supplementation, influences the incidence of neural tube defects and to identify new therapies to correct folate-resistant neural tube defects. Folic acid supplementation prior to conception has been shown to reduce the incidence of NTDs in humans by up to 70%. However, the mechanisms by which folic acid reduces the occurrence of NTDs is unknown. We are testing our mouse models of NTDs to better understand how folic acid interacts with the genetic components of neural tube closure.

Sponsored by La Trobe Institute for Molecular Science

PLE-FRI-20

CHAPERONE-ASSISTED PROTEIN FOLDING IN HEALTH AND DISEASE

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Many proteins depend on molecular chaperones for efficient folding within the crowded environment of the cell. Assistance of protein folding is provided by several types of chaperone which act to prevent misfolding and aggregation, often in an ATP-dependent mechanism. In the cytosol, nascent chain-binding chaperones, including trigger factor and Hsp70, stabilize elongating chains on ribosomes in a non-aggregated state. Folding is then achieved either on controlled chain release from these factors or following polypeptide transfer to downstream chaperones, such as the chaperonin GroEL, which provides a cage-like nano-compartment for single protein molecules to fold in isolation. Recent work indicates that these folding cages not only function to prevent aggregation but also to modulate the energy landscape of the folding process, thereby accelerating folding for certain proteins. The cytosolic chaperone machinery is also important in controlling protein misfolding and aggregation in the context of neurodegenerative disorders, such as Parkinson's and Huntington's disease (HD). Specifically, Hsp70 and chaperonins can cooperate to prevent the formation of toxic protein oligomers, as shown for the HD protein. A reduction in the general capacity of the chaperone system during aging may be critical in the manifestation of these late-onset diseases, suggesting up-regulation of chaperones as a possible therapeutic strategy.

PLE-FRI-21**SMALL RNAS, VIRAL COUNTER DEFENCE AND MOBILE SILENCING SIGNALS****Waterhouse P.M.**

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RNA interference (RNAi) is widely used to silence genes in plants and animals. RNAi is induced by delivery of exogenous dsRNA or self-complementary hairpin (hp) RNA, which is processed by Dicer-like (DCL) enzymes into ~21nt short interfering (si) RNAs. These siRNAs guide the degradation of target mRNA by endonuclease complexes. A similar, endogenously-triggered process regulates the expression of some developmental genes through ~21nt micro (mi) RNAs. Plants have four main types of DCL and three of them, DCL2, 3 and 4, process both replicating viral RNAs and RNAi-inducing hpRNAs into 22, 24 and 21nt siRNAs indicating that RNAi in plants operates through the viral defence pathway. In contrast, DCL1 processes miRNAs in plants and has little or no role in RNAi. Endogenous small (21-24nt) RNAs have important functions in controlling gene expression, chromatin status, and defence against viruses. In plants, these small RNAs are produced by a matrix of interactions involving 4 different Dicer proteins and 5 different dsRNA-binding (DRB) proteins. We are studying these interactions and elucidating their roles in the different pathways. We are also studying viral counter-defence and a mobile signal which induces the silencing of genes in distal regions of the plant.

PLE-FRI-22

EDUCATION OF UNDERGRADUATE AND GRADUATE STUDENTS IN THE BIOMEDICAL SCIENCES – EDUCATION FOR WHAT?**Lodish H.F.^{1,2}**¹Whitehead Institute for Biomedical Research. ²Massachusetts Institute of Technology.

For many years I have taught undergraduate courses at MIT in Cell Biology and Biotechnology, and I direct a research laboratory of some 25 scientists at all levels. I have trained over 200 graduate students and postdoctoral fellows, and about 70% have had academic careers, many with great distinction. But most of our current students and fellows will work outside of academia – in biotech or pharmaceutical companies, in publishing, in patent law, or in public policy. I have been fortunate to have participated actively in all of these areas and I have become cognizant of how poorly we prepare our students and fellows for careers outside academia. I've served, for instance, as an expert witness in five very high profile and high stakes patent trials. And we are all asked to patent our discoveries so our research institutes and companies can make money. Yet the vast majority of scientists have only a vague notion of what a patent is or what it protects, and even fewer know the parts of a patent. And very few faculty are able to advise students who wish to go into patent law as a career, or for that matter into any career outside academia. Currently I chair the Scientific Advisory Board of the Massachusetts Life Sciences Center (a Board I convinced the Governor to establish) that oversees Massachusetts' 10 year \$1 billion investment in the Life Sciences. Most of the funding goes to very early small companies with good ideas but little cash. How can we mentor our students to start a company, or to work in venture capital? How can we train our students to work in biotech or pharmaceutical companies when most university faculty have never visited companies and have little idea how they function? Or what backgrounds the various jobs in companies require? Perhaps, having started six companies, some very successful and some not, I have some insights to share. And how do advise our students who may want to work in areas such as science policy? (Convincing a State Legislature to appropriate millions of dollars a year for life sciences companies is not easy.) Quite deliberately, many of my research fellows have an MD degree, and they definitely have helped focus some research in my lab on medical problems. This is an attempt to remedy a major failing of our current system of undergraduate and graduate education in the life sciences - we are supposedly training our students to conduct research that ultimately will lead to new diagnostics or therapies or devices for human disease, but they are given only the most rudimentary understanding of human disease and how they are actually treated. Increasingly our students learn little about human biology or physiology as well; this limits the utility of the research they ultimately conduct. As universities and hospitals are now trying to edge their laboratory activities into "translational research" most faculty are simply untrained and unable to participate in these more applied areas. Success in any branch of science depends on an ability to write papers and grants clearly and concisely, yet few of us are able to teach our students how to write a paper. Many would like a career in academic publishing or science reporting but few academics have any experience in these areas. Perhaps, from writing and organizing several editions of a cell biology textbook I have some words of wisdom to share about these careers. Finally, modern biomedical science requires the active participation of computer scientists, mathematicians, physicists, electrical and mechanical and chemical engineers, and many others. How do we transmit enough molecular and cell biology and pathophysiology to these folks so that they can actively participate in this research? These are not easy questions and there are many possible answers, but without first identifying the problems it's unlikely we'll come up with good long- term solutions.

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ABA BIOSYNTHESIS AND SIGNALING

Zhu J.-K.

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The phytohormone abscisic acid (ABA) is a central regulator of plant responses to abiotic stresses such as drought and soil salinity. Under osmotic stress imposed by drought or salinity, cellular ABA content increases dramatically. Significant progress has been made in the elucidation of ABA biosynthesis and signaling. However, major gaps remain in our understanding of how osmotic stress is sensed to induce ABA production. I will review recent breakthroughs in the identification and characterization of ABA receptors and downstream signaling pathways, and present new data on osmotic stress regulation of ABA biosynthesis.

