PLENARY LECTURES

Monday - Wednesday

PLE-MON-01

SORTING OF SMALL RNAS INTO EXTRACELLULAR VESICLES SECRETED BY HUMAN CELLS

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Highly purified extracellular vesicles (EVs) isolated from human cell lines display a small number of substantially (~ 1000 fold) enriched miRNAs that differ from one cell line to another. In spite of the small number of such species, no single RNA sorting sequence is evident. In order to explore the mechanism of RNA sorting, we established a cell-free reaction that reproduces the selective incorporation of synthetic, mature miRNAs (miR223 and miR122) into vesicles formed in a reaction containing membranes and cytosol from mechanically disrupted HEK293 cells. The sorting reaction requires both membrane and cytosol and is stimulated by hydrolysable ATP and incubation at a physiologic temperature. Using biotinlyated derivatives of two different miRNAs, we found different sets of RNA binding proteins incorporated along with each species, among which the proteins Ybx1 and Lupus La are required to sort mir223 and miR122, respectively. EVs also contain more abundant major species of small RNA including full-length tRNA, Y-RNA and vault RNA, and each requires the Ybx1 protein for selective sorting into exosomes secreted by cells and into vesicles in the cell-free reaction. tRNAs in EVs appear to have a distinct chemical modification that is much less abundant in tRNAs in HEK293 cells. This modification may be involved in tRNA sorting or in the function of tRNA transported to a target cell.

Shurtleff MJ, Temoche-Diaz MM, Karfilis KV, Ri S, Schekman R (2016). Y-box protein 1 is required to sort microRNAs into exosomes in cells and in a cell-free reaction. Elife. Aug 25;5. pii: e19276. doi: 10.7554/eLife.19276. PMID: 27559612
Shurtleff, M., Yao, J., Qin, Y, Nottingham, R., Temoche-Diaz, M., Schekman, R and Lambowitz, A. (2017) A broad role for YBX1 in defining the small non-coding RNA composition of exosomes PNAS 2017 October, 114 (43) E8987-E8995. https://doi.org/10.1073/pnas.1712108114
Shurtleff, M., Temoche-Diaz, M. and Schekman, R. (2018) Extracellular Vesicles and Cancer: Caveat Lector. Ann. Rev. Cancer Biology https://doi.org/10.1146/annurev-cancerbio-030617-050519.

PLE-MON-02

THE PHASE OF FAT: MECHANISMS AND PHYSIOLOGY OF LIPID STORAGE

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All organisms face fluctuations in the availability and need for metabolic energy. To buffer these fluctuations, cells use neutral lipids, such as triglycerides, as energy stores. We study how lipids are stored as neutral lipids in cytosolic lipid droplet organelles. Specifically, we investigate the molecular processes that govern the synthesis of energy storage lipids as well as their storage in and mobilization from lipid droplets. In modern societies, individuals often face metabolic energy excess, leading to metabolic diseases. We will present our studies on the causal link between energy excess and metabolic disease.

PLE-MON-03

MAKING A PORE: SIGNALING AND TRANSCRIPTION FACTOR CONTROL OF STOMATAL DIFFERENTIATION

Torii K.U.[1, 2]
[1]Investigator, Howard Hughes Medical Institute, Department of Biology, University of Washington. [2]Principal Investigator, Institute of Transformative Biomolecules, Nagoya University.

Multi-cellular organisms must coordinate cell behaviors to achieve a functional form. For plants, the cell walls present a special challenge, as cell movement is limited and patterns must emerge through controlled cell proliferation and differentiation. Our group defining the molecular mechanism specifying patterning using the development of specialized cell structures called stomata as a model. Stomata, cellular valves on the plant epidermis, serve as critical interface between plant and atmosphere, and the presence of stomata impacts global carbon and water cycles. For this reason, proper stomatal development and function are critical for plant growth and survival, especially in light of changing climate. Focusing on stomatal development, where the initiation, proliferation and differentiation of a bipotent precursor cell can be monitored at a single cell resolution, we aim to elucidate the complex interplay of cell-cell signaling and master regulatory transcription factors specifying tissue patterning. In this seminar, I will present our recent findings revealing the structural insight into the stomatal fate decision, and the molecular circuitry creating stomata.

PLE-MON-04

PROBING THE STRUCTURAL DETAILS OF ION-CHANNEL FUNCTION USING VENOM PEPTIDES

Mobli M.
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Ion channels and their structural relatives comprise one of the largest superfamilies of signal transduction proteins. Unsurprisingly, these ion channels are also among the most common drug targets. Ion channels, however, have proven particularly recalcitrant to traditional drug discovery approaches and our work seeks to address this through improved understanding of the structure and function of these channels.[1] Disulfide rich venom peptides are known to include molecules that modulate the activity of ion channels by unique allosteric mechanisms.[2] These peptides, provide an excellent opportunity to study the structural details of channel inhibition for drug development. I will present work from our group that overcome difficulties in the production and characterisation of these cysteine stabilised peptides, allowing them to be used for probing channel structure and function.[3] Our group is currently also developing methods to stabilise the ligand binding domain of the membrane embedded ion-channels in solution using lipid nanodiscs for high-resolution structural studies by nuclear magnetic resonance (NMR) spectroscopy and high-throughput screening assays, capable of identifying weak allosteric modulators. In combination with existing cell-based assays it is anticipated that this will provide a new approach to identifying drug candidates in a field that has proven challenging for drug development. **References:** 1. Zhang, A.; Sharma, G.; Undheim, E. A. B.; Jia, X.; Mobli, M., A complicated complex: ion channels, voltage sensing, cell membranes and peptide inhibitors. Neuroscience Letters 2018, In press (accepted 2018-01-11). 2. Mobli, M.; Undheim, E. A. B.; Rash, L. D., Modulation of Ion Channels by Cysteine-Rich Peptides: From Sequence to Structure. In Advances in Pharmacology, Geraghty, D. P.; Rash, L. D., Eds. Academic Press: 2017; Vol. 79, pp 199-223. 3. Miljenovic, T. M.; Jia, X.; Mobli, M., Nonuniform Sampling in Biomolecular NMR. In Modern Magnetic Resonance, Webb, G. A., Ed. Springer International Publishing: Cham, 2018; pp 2035-2054.

PLE-MON-05

PLANT-MICROBE SYMBIOSES: HORMONES AND THE ART OF SELF-CONTROL

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To optomise plant growth to meet the demands of a rapidly changing world, we must understand how plants maximise nutrient uptake. In modern agriculture, application of artificial fertilisers is widely used to promote plant growth, with unintended negative consequences for the environment. Another strategy that plants have to gain access to nutrients is the formation of specialised partnerships (symbioses) with soil microbes. In these intimate associations, the microbes enter the plant root and provide previously inaccessible nutrients (nitrogen and phosphorous) in exchange for plant carbon. Leguminous plants such as peas, beans and lentils can host both bacterial and fungal symbionts in the root simultaneously, meaning a particularly delicate balance must be struck to ensure that the nutrient requirements of the plant are met most efficiently. Plant hormones are a discreet set of small molecules with potent effects on plant development. In our work, we examine the role of plant hormones in symbioses using our extensive collection of hormone-related pea mutants and through the measurement of minute quantities of these hormones. This includes a particular focus on how hormones, working alone and together, influence the development of the root to successfully accommodate the microbial partners.

PLE-MON-06

GENE EDITING APPROACHES TO DEVELOP ANTI-HIV THERAPIES

Cannon P.
University of Southern California.

Gene editing uses targeted nucleases such as CRISPR/Cas9 to create sequence-specific breaks in DNA, whose subsequent repair is then exploited to achieve different genetic outcomes. If the cell's error-prone non-homologous end joining (NHEJ) pathway is used to repair the DNA break, insertions or deletions (indels) can also be introduced that lead to gene disruption. In contrast, homology directed repair (HDR) pathways allow for more precise repair, since they copy genetic information from a homologous sequence. However, this pathway can be hijacked by introducing an exogenous DNA template into the cell, and thereby direct a specific DNA mutation, or promote the site-specific insertion of a larger DNA cassette. My lab works to develop gene editing technologies and in particular for use in hematopoietic stem cells (HSC). Much of our work has focused on HIV, whose life-cycle involves its integration into the chromosome of a target cell, where the virus becomes a permanent genetic passenger. HIV replication can be controlled by life-long adherence to antiretroviral drugs, but cannot currently be cured, and gene editing technologies are being considered as novel strategies to fight this disease. For example, NHEJ-mediated gene knockout can disrupt the CCR5 co-receptor, and a clinical trial is underway to evaluate this approach. In addition, the integrated HIV genome itself is also considered a possible target for NEHJ-mediated disruption. Finally, certain host genes can be edited to acquire gain-of-function mutations that enhance their anti-HIV activity. I will discuss gene editing in general, and use the example of HIV to show the potential - and the challenges - for this new class of gene therapeutics.

PLE-MON-07

REAL ENZYMES AND HOW THEY'VE CHANGED MY VIEW OF LIFE

Patrick W.M.
School of Biological Sciences, Victoria University of Wellington, New Zealand.

When I was a PhD student, I was heavily influenced by enzymologists such as Knowles and Wolfenden, who emphasised the supreme catalytic power of enzymes. The goal of every wannabe protein engineer was to design or evolve a novel enzyme with kinetic parameters to rival triose phosphate isomerase or ornithine decarboxylase. This goal is certainly laudable, but after decades of increasingly sophisticated method development, few – if any -- of us have ever achieved it. Will we ever?

In this talk, I will describe our recent work to characterise non-model enzymes from non-model organisms. It turns out that enzymologists have been studying a rather biased subset. Most of the enzymes on Earth are slow and sloppy. Excitedly, that makes us all better protein engineers than we thought!

PLE-TUE-08

THE CELL BIOLOGY AND BIOCHEMISTRY OF MICROTUBULE NUCLEATION

Brouhard G.J.
McGill University.

Microtubules are born and reborn continuously, even during quiescence. These polymers are nucleated from templates, namely γ-tubulin ring complexes (γ-TuRCs) and severed microtubule ends. Interestingly, the rate of microtubule nucleation increases as cells enter mitosis, and all cells nucleate microtubules in distinct regions of their cytoplasms. How are these spatial and temporal profiles for microtubule nucleation established? One hypothesis is based on the observation that the rate of microtubule nucleation is strongly affected by both catastrophe factors and anti-catastrophe factors, suggesting an essential relationship between nucleation and catastrophe. Alternatively, the rate of nucleation may be related to the kinetics of microtubule growth and modulated by microtubule polymerases. We do not understand the relative importance of catastrophes versus kinetics in determining a cell's nucleation profiles. I will discuss my lab's recent attempts to address this knowledge gap using a mix of single-molecule biophysics, cell biology, and structural biology.

PLE-TUE-09

MECHANOSENSING AT THE SURFACE: SIGNALING MECHANISMS IN MAMMALIAN TOUCH

Lumpkin E.A.
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My group studies genes, cells and signals underlying the sensations of touch, pain and itch. Our research has unveiled how mechanosensitive epithelial cells work in concert with the nervous system to generate different qualities of touch sensation. We have identified distinct sensory functions of epithelial Merkel cells using optogenetics, neurophysiology, intersectional mouse models and molecular approaches. Current studies are defining molecular mechanisms of cell-cell signaling between epithelia and neurons, unravelling conserved functions of mechanoreceptors across tissues, and elucidating mechanisms that establish and maintain epithelial-neuronal connections during development.

PLE-TUE-10

CELL CYCLE REGULATION OF DIFFERENTIATION IN DEVELOPMENT AND DISEASE

Philpott A.
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It is essential that division and differentiation are carefully co-ordinated during development. Moreover, a disruption of this co-ordination is a central characteristic of many forms of cancer. We are investigating the molecular mechanisms by which the cell cycle machinery can directly control the activity of key transcription activators, the proneural proteins, which are responsible for regulating differentiation in the nervous system, pancreas, gut and multiple other tissues. We see that chromatin binding and transcriptional activity of basic helix-loop-helix transcription factors of the “proneural” family, which regulate fate choice and differentiation in many tissues, is regulated by multi-site phosphorylation by cell cycle kinases. Inhibiting proneural protein phosphorylation preferentially activates targets associated with driving cell cycle exit and differentiation, and this is key to altering the balance away from maintenance of a proliferative state. Our results lead us to a model whereby proneural transcription factor post-translational modification intersects directly with the epigenetic landscape of downstream targets to determine whether cells maintain their progenitor status or undergo differentiation during development and in adult homeostasis in multiple tissues. For instance supporting this model using engineered, we see that post-translational modification of proneural proteins controls both the formation of endocrine cells in the developing pancreas and the ability of intestinal secretory progenitor cells to respond to tissue injury by contributing to repair. We are also investigating how proneural protein phosphorylation by sustained cdk activity may result in a fundamental shift in the behavior of cancer cells, disabling their ability to undergo mitotic arrest and terminal differentiation. In particular, the paediatric cancer neuroblastoma is associated with stalling of normal differentiation resulting in excessive proliferation of neuroblastic precursors of the sympathetic nervous system. We see that CDK-dependent phosphorylation of the proneural protein ASCL1 in in neuroblastoma plays a critical role in controlling the balance between cell proliferation and differentiation; preventing CDK-dependent phosphorylation of ASCL1 results in changes in the genome-wide transcriptional programme of neuroblastoma cells, leading to suppression of pro-proliferative targets and simultaneous activation of genes that drive cell cycle exit and differentiation. Mechanistically, ASCL1 ChIPSeq reveals that dephosphorylation of Ascl1 leads to a disabling of the super-enhancer network that supports the progenitor state and enhanced binding of un(der)phosphorylated ASCL1 at sites associated with pro-differentiation targets. Finally, we also show that chemical CDK inhibition is sufficient to drive differentiation of neuroblastoma cells in a manner dependent on endogenous ASCL1. Therefore, we conclude that CDK-dependent phosphorylation of ASCL1 acts as a critical fulcrum controlling the balance between proliferation and differentiation and thus points to novel therapies for neuroblastoma. Furthermore, post-translational control of may play a key role in regulation of other proneural proteins that have been shown to act as lineage-specific oncogenes in a number of settings.

PLE-TUE-11

FUNCTIONAL GENOMICS OF SYMBIOTIC NITROGEN FIXATION IN LEGUMES

Udvardi M., Roy S., Liu W., Nova Franco B., Espinoza M., Kang Y., Torres-Jerez I. and Huertas R.
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Discovery of the first plant gene required for legume nodule development and symbiotic nitrogen fixation (SNF), *LjNIN* from *Lotus japonicus*, occured in 1999 (Schauser et al, 1999). Today, over 150 genes in multiple legume species have been found to be required for nodule development and/or effective nitrogen fixation, via forward-genetics or by genomics-informed reverse-genetics (Roy, Liu, et al., unpublished). These genes have been implicated in signaling between rhizobia and legumes, infection and accommodation of the micro-symbiont in plant cells, nodule organogenesis, and plant metabolism in support of bacterial nitrogen fixation. Although some of these genes appear to be indispensable for SNF in several legumes species, not all are necessary in all species. This may reflect genetic and/or other functional redundancy within species related to either ancient genome duplications or more recent tandem duplications of genes. It also appears to reflect, to some extent, distinct co-evolution of legumes and specific rhizobia. Despite the large number of genes now known to be required for SNF in legumes, our knowledge of the molecular and cellular basis of nodule development and metabolism remains fragmentary. This talk will summarize what is known about the genetics of the various processes that lead to and support SNF, including our work on *Medicago truncatula*, and highlight some of the gaps in our knowledge in these areas. Finally, we address the question: how can SNF be improved in legumes with so many genes implicated? We are using natural variation in SNF in approximately 200 ecotypes of *M. truncatula* to identify genes that contribute to effectiveness in this species, via genome-wide association studies, with a view to developing plant breeding strategies to improve SNF in crop legumes. Examples of putative SNF effectiveness genes will be presented.

PLE-TUE-12

IMMUNE SENSING OF NON-PEPTIDIC ANTIGENS

Rossjohn J.[1, 2, 3]
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Prof. Jamie Rossjohn FAA FAHMS FLSW FMedSci, Australian Research Council Laureate Fellow. The immune system is key to our survival as the human population is constantly under threat from devastating pathogens. Humans defend themselves from microbes by mounting protective immune responses that include activating the innate and adaptive arms of the cellular immune system. Conversely, while cellular immunity is critical to our survival, immune dysfunction is a major contributor to disease burden globally. Major advances in understanding the immune system are beginning to impact on human health through novel immunotherapies. Nonetheless, there are many aspects of human immunity we do not understand. T cells, via their T cell receptor (TCR) expressed on its cell surface, play a critical role in mediating cellular immunity. While is generally considered that TCRs interacts with peptide antigens bound to the Major Histocompatibility Complex (MHC), TCRs also interact with lipid-based Ags bound by CD1 family members. Using a combination of chemical, structural and immunological approaches, we have also established how T cells, termed MAIT cells, can recognise microbial-based vitamin B metabolites. I shall detail the structural basis of immune sensing of non-peptidic antigens.

PLE-TUE-13

CELL MEMBRANE WATER CHANNELS WITH BUILT IN ION CHANNELS

Byrt C.S.[1, 2], Qiu J.[1, 2], McGaughey S.[1, 2], Groszmann M.[3, 4], Bose J.[1, 2] and Tyerman S.D.[1, 2]
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Cell function is dependent on maintenance of water and ionic homeostasis. Aquaporins are regulated by cells to achieve water homeostasis, but in addition they may also be required for ion homeostasis. Cell water permeability is determined by the water conductance and density of aquaporins present in the plasma membrane. Plants express in the order of 30 to 70 different types of aquaporins, depending on the plant species, and these include a group called PIPs that are particularly abundant in plasma membranes. PIPs generally form tetramers with each monomer capable of allowing the passage of water. There are subsets of PIP tetramers that allow passage of other solutes, and we have identified PIPs that can change between functioning as water channels and non-selective cation channels (NSCCs) when expressed in heterologous systems. In plants there are likely to be multiple types of NSCCs and previous studies have revealed that NSCCs, for which the molecular candidates are so far unidentified, can provide a pathway for nutrient transport, and also for sodium transport under salinity stress. PIPs functioning as ion channels can allow passage of sodium and potassium, and they share similar properties with previously reported NSCCs. For example, NSCC and PIP ionic conductance are sensitive to calcium, pH and cyclic nucleotides. We are testing whether PIPs can account for any of the previously observed NSCC functions in plants by studying ion transport traits in mutant and transgenic lines of Arabidopsis where the PIPs of interest are either knocked out, overexpressed or mutated to change their ion channel function. Testing whether PIPs are implicated in maintaining water and ionic homeostasis in plants is an important step towards resolving the roles of PIPs in plant tolerance to dry, saline and nutrient deficient environments.

PLE-TUE-14

INVESTIGATING HOW GENETIC AND ENVIRONMENTAL FACTORS DISRUPT MAMMALIAN EMBRYOGENESIS

Dunwoodie S.L.
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Congenital malformations arise due to genetic and environmental factors and understanding the interplay between these in causing malformation might lead to preventative opportunities, in some cases. We are identifying genetic and environmental factor that disrupt embryogenesis in human and mouse. In mice, we have shown that short-term gestational hypoxia disrupts progenitor cell populations in embryos, leading to vertebral and cardiac defects that are commonly found in humans. We showed that hypoxia induced the unfolded protein response (UPR) and in doing so inhibited cap-dependent translation, which resulted in the loss of fibroblast growth factor signal transduction in progenitor cells in the presomitic mesoderm and the second heart field. We propose that many environmental risk factors for congenital malformation in humans induce the UPR and that this might be a unifying mechanism leading to disrupted embryogenesis (Sparrow et al Cell 2012; Shi et al Development 2016). In humans studying families with vertebral and cardiac defects we identified mutations in four cases in either of two genes: *3-hydroxyanthranilic acid 3,4-dioxygenase (HAAO)* and *kynureninase (KYNU)*, encoding kynurenine pathway enzymes. All four patients had vertebral, cardiac and renal defects, amongst others and recurrent miscarriage was a feature in two families. Nicotinamide adenine dinucleotide (NAD) is synthesized de novo from tryptophan via the kynurenine pathway. NAD is also synthesized more directly from vitamin B3. The patients had reduced circulating NAD levels. Haao or Kynu null mouse embryos developed similar defects to the patients, due to NAD deficiency. In null mice NAD deficiency, malformations and miscarriage were prevented by niacin (vitamin B3) supplementation during gestation (Shi et al NEJM 2017).

PLE-WED-15

HOW IS ELECTRICAL SIGNAL GENERATED? STRUCTURAL AND MECHANISTIC INVESTIGATIONS OF NA_V CHANNELS

Yan N.
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The voltage-gated sodium (Na_v) channels are responsible for the initiation and propagation of action potentials. Being associated with a variety of channelopathies, they are targeted by multiple pharmaceutical drugs and natural toxins. We determined the crystal structure of a bacterial Na_v channel Na_vRh in a potentially inactivated state a few years ago, which is a homotetramer in primary sequence but exhibits structural asymmetry. Employing the modern methods of cryo-EM, we recently determined the near atomic resolution structures of a Na_v channel from American cockroach (designated Na_vPaS) and from electric eel (designated $EeNa_v1.4$). These structures reveal the folding principle and structural details of the single-chain eukaryotic Na_v channels that are distinct from homotetrameric voltage-gated ion channels. Unexpectedly, the two structures were captured in drastically different states. Whereas the structure of Na_vPaS has a closed pore and four VSDs in distinct conformations, that of $EeNa_v1.4$ is open at the intracelluar gate with VSDs exhibiting similar “up”states. The most striking conformational differenc occurs to the III-IV linker, which is essential for fast inactivation. The III-IV undergoes a pronounced repositioning from Na_vPaS to $EeNa_v1.4$, resulting in the insertion of the IFM fast inactivation motif on the III-IV linker into the corner enclosed by the S4-S5 and S6 segments in repeats III and IV of $EeNa_v1.4$. Based on the structural features, we suggest an allosteric blocking mechanism for fast inactivation of Na_v channels by the IFM motif. Structural comparison of the conformationally distinct $EeNa_v1.4$ and Na_vPaS provides important insights into the electromechanical coupling mechanism of Na_v channels.

PLE-WED-16

CYTOKININ: A HORMONE WITH MANY ROLES

Jameson P.E.
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In the early 1980's, the cytokinins were declared to be "the hormone without a role". Now the cytokinins are known to be involved in most aspects of the plant life cycle. Early work showed that radio-labelled cytokinins travelled from the roots to the shoots. Our use of a root-specific Cu-inducible promoter driving cytokinin biosynthesis (via *IPT*) confirmed this mobility, as well as a role for cytokinin in releasing axillary buds. Further, we recently showed that root-produced cytokinins play a role in signalling the nitrogen status of the plant and suggest that the cytokinins are involved not only in the uptake of nitrogen but also in the homeostatic mechanism balancing uptake and assimilation of nitrogen with availability of carbohydrate in perennial ryegrass. During reproduction, cytokinin is limiting to pod/seed number. In pea as in arabidopsis, tipping the balance in favour of accumulation of cytokinin (by reducing cytokinin destruction) in the shoot apical meristem may hold the key to pod and seed number. Moreover, root-supplied cytokinin remains restricted to the maternal tissue and does not cross to filial tissue, as seeds form. Our gene expression studies confirmed that cytokinin is biosynthesised within the developing seed, and that there is a complex interplay between biosynthesis, metabolism and destruction during seed development, along with differential activity of various homoeologous gene family members in both tetraploid forage brassica and hexaploid wheat. Such detailed knowledge is needed to guide future gene editing for yield improvement. While cytokinins are intimately linked to cell division in cereals, in legumes they are implicated in enhancing sink activity. In double transgenic peas ectopically expressing both an amino acid transporter (*AAP*) and a sucrose transporter (*SUT*), we showed both elevated *IPT* expression and elevated endogenous cytokinins in the seed coat, alongside increased yield, clearly indicating, again, the intimate relationship between cytokinin, nitrogen and carbohydrate. The ability of cytokinins to delay senescence has been known for many years, as has the metabolism and/or the inactivation of cytokinin in senescing leaves. Unexpectedly, we have shown cytokinin biosynthesis increasing in both mature kiwifruit and in senescing leaves, and suggest that the free radical scavenging abilities of cytokinin may be associated with maintenance of mitochondrial membranes late into the senescence process. Several gall-forming bacteria produce cytokinins. However, for many years the multiple shoots and leafy galls produced by *Rhodococcus fascians* have been an enigma, because of the lack of elevated cytokinin in infected tissue. Both epiphytic and endophytic *R. fascians* enhance transporter gene expression but only the endophytic strains markedly affect morphology. We recently confirmed that endophytic *R. fascians* strains can produce a novel methylated cytokinin, and suggest that the interaction between this cytokinin, the new family of sugar transporters (SWEETS) and cell wall invertases enhances the multiple shoots formed following seed inoculation of pea by endophytic strains of *R. fascians*. The cytokinins are clearly a hormone with many roles.

PLE-WED-17

AN EXPANDING JOB DESCRIPTION FOR THE ZINC FINGER TRANSCRIPTIONAL REPRESSOR BLIMP1/PRDM1

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The zinc finger transcriptional repressor B-lymphocyte-induced maturation protein 1 (Blimp1), encoded by the *Prdm1* gene, originally cloned as negative regulator of β-interferon gene expression and subsequently identified as a master regulator of plasma cell terminal differentiation, governs cell fate decisions in the developing embryo and adult tissues. In the early embryo, Blimp1 is required to specify the primordial germ cell lineage. Loss of function mutant embryos die at mid-gestation due to defective placental morphogenesis. Conditional deletion experiments demonstrate that Blimp1 activities are also essential for development of the pharynx, forelimbs and heart. Blimp1 also regulates the developmental switch responsible for post-natal reprogramming of the intestinal epithelium. Most recently we discovered that Blimp1 identifies a rare population of luminal stem cells essential for mammary gland morphogenesis and organ homeostasis. Via its dynamic patterns of expression, associations with diverse epigenetic partners and widespread recognition of genomic target sites Blimp1 plays an essential role controlling developmental programmes in multiple tissue contexts.

PLE-WED-18

EXTRINSIC AND INTRINSIC FORCE REGULATES CANCER PROGRESSION, AGGRESSION AND TREATMENT

Weaver V. and Colleagues
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All cells experience force and possess mechanosensory mechanisms that enable them to detect mechanical stimuli and transduce these cues into biochemical signals that modify protein function and alter gene expression to influence cellular behavior. Tumors have higher cell and tissue level forces and transformed cells exhibit perturbed mechanosensing. We have been studying the genesis of the altered tumor cell and tissue force and how cells sense and transduce mechanical cues to drive tumor formation and aggression and whether and how this influences treatment response. Using an array of in vitro and in vivo models we found that the ECM progressively stiffens in peripheral tumors such as the breast, skin and pancreas mediated largely by increased collagen deposition, remodeling and crosslinking and induction of fibrosis. Even in tumors such as glioblastomas, which do not exhibit collagenous fibrosis the ECM stiffens due to enhanced hyaluronic acid deposition and proteoglycan crosslinking. We consistently find that a stiffened tumor ECM enhances integrin signaling to promote malignant transformation and tumor aggression that ultimately compromise treatment responsiveness. Consistently, inducing ECM tension or increasing integrin signaling promotes the malignant transformation of pre malignant oncogenically-primed cells and drives the aggressiveness of tumors, whereas inhibiting ECM stiffening prevents tumor progression and reduces aggression. Importantly, when tumor cells are oncogenically transformed or loose expression of critical tumor suppressors they increase their actomyosin tension and enhance their integrin focal adhesion and growth factor receptor signaling. The high tumor cell tension fosters tumor progression and aggression in part by stiffening and remodeling the ECM. The stiff ECM also compromises the tissue vasculature to induce hypoxia and HIF1a to promote a mesenchymal-like frequently metastatic phenotype that is highly resistant to therapy. The stiff ECM also modulates tumor immunity and regulates levels of key repressors that modulate anti-tumor cytotoxic responsiveness.

PLE-WED-19

A GADD45α-ING1-C/EBP AXIS REGULATES ENERGY HOMEOSTASIS AND ORGANISMAL AGING

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Changes in DNA methylation are among the best-documented epigenetic alterations, which accompany aging. However, if and how altered DNA methylation is causally involved in aging has remained elusive. GADD45α and ING1 are adapter proteins for site-specific demethylation by TET methylcytosine dioxygenases. We show that *Gadd45a/Ing1* double knockout mice (DKO) display premature aging and phenocopy impaired energy homeostasis and lipodystrophy, characteristic of Cebp (CCAAT/enhancer binding protein) mutants. Correspondingly, GADD45α occupies C/EBPβ/δ-dependent super-enhancers, and cooperatively with ING1 promotes local DNA demethylation to permit C/EBPβ recruitment. Our study reveals a causal nexus between DNA demethylation, metabolism and organismal aging.