Euchromatic Histone Methyltransferase1 tethers heterochromatin to the nuclear periphery.

ShravantiRampalli
Centre for Inflammation and Tissue Homeostasis, inStem
shravantird@instem.res.in

H3K9me2 methyl mark deposited by Euchromatic histone methyltransferases (EHMT's) serves as critical signal for perinuclear anchoring of heterochromatin. However, there is no direct evidence that these proteins are indeed required for attachment of heterochromatin to nuclear periphery. Here, we uncovered the novel role of Ehmt's in tethering of heterochromatin to nuclear lamina. In search for mechanism we identified Ehmt's associates and methylate with nuclear lamins. I will discuss how EHMT mediated pleotropic effects that influences heterochromatin organization and lamin methylation impacts fundamental changes associated with the intrinsic aging process.

Dynamic expression of tRNA-derived small RNAs define cell states

Dasaradhi Palakodeti
Technologies for the Advancement of Science, inStem
dasaradhip@instem.res.in

Transitions from a stem to differentiated state require precise control of gene expression. In the current study, we explored the novel role of tRNA derived small RNAs (tsRNAs) in regulating the translation critical for transition of embryonic stem cell to a differentiated state. Small RNA profiling from mouse embryonic stem cells (ESCs) and retinoic acid (RA) induced differentiating ESCs identified tsRNAs that are enriched specifically during early differentiation stages. Biochemical and functional studies demonstrated the role of tsRNAs in fine-tuning differentiation and maintenance of stemness by suppressing translation. Transcriptome analysis of mRNA associated with tsRNAs revealed selective state-dependent association of tsRNAs with transcripts, critical for differentiation and lineage commitment. Our study also suggested that the specificity of tsRNA to particular mRNA could be mediated by preferential binding of tsRNAs with specific RNA binding proteins and ribosomal proteins. Together, we propose a role for tsRNAs in translational control of specific pools of mRNA to facilitate efficient cellular differentiation.

Making commitments: how key metabolites determine cell proliferation decisions

Sunil Laxman
Regulation of Cell Fate, inStem
sunil@instem.res.in

It is now apparent that the cellular metabolic state, exemplified by key metabolites, can determine cell fate. However, metabolism is often misunderstood by researchers and educators alike, who view it as a series of arbitrary accidents, instead of a series of elegant, interconnected and balanced processes that are the optimal route to an outcome. In my talk, I will put out a perspective of how metabolism works through fairly simple rules that can be intuitively understood, by attempting to understand what a cell is trying to achieve. I will illustrate this with two examples. In the first example, I will provide a metabolic and mechanistic explanation for what makes methionine a special molecule, showing how methionine availability drives a global metabolic transformation and strong commitment to cell proliferation. In the second example, using yeast metabolic oscillations as an illustrative system, I will offer a theoretical model framework to understand and predict how the availability of acetyl-CoA will determine commitments to the quiescent vs proliferative state.

Role of mechanical signaling in maintaining stem cell quiescence in mouse skin

Srikala Raghavan
Centre for Inflammation and Tissue Homeostasis, inStem, srikala@instem.res.in

Vinculin, a mechano-coupling protein, links the actin cytoskeleton to the cell-substratum and cell-cell junctions. It docks focal adhesion partners and alpha-actinin at cell-cell junctions, and regulates mechanically induced signaling pathways. Vinculin knock-outs in mouse are embryonic lethal due to defects in cell adhesion and migration. Vinculin is variably expressed in bulge stem cells of the hair follicle, which is the main pool of quiescent stem cells in the skin. We studied the role of vinculin in mouse skin by generating a conditional K14 driven knock out in skin epidermis. The vinculin knockout mice displayed loss of hair and acceleration of the hair follicle cycle, without complete hair loss. Pulse-chase experiments performed with BrdU revealed that the bulge stem cells fail to maintain their quiescence in the KO. We are now addressing how the loss of vinculin, a mechano-transducer results in loss of stem cell quiescence, as well as the role played by the stem cell niche. This study will clarify underlying mechanism affecting the normal hair follicle cycle, and the signaling required to maintain hair follicle stem cell quiescence.

Notch1 activity regulates survival and lineage integrity in T-regulatory cells

Apurva Sarin
Regulation of Cell Fate, inStem
sarina@instem.res.in

Lymphocyte differentiation is essential for their function and survival in diverse niches. In T-lymphocytes, differentiation is triggered by receptor signaling, requires transcription factors and cytokines, as well as the engagement of specific metabolic programs. How these are coordinated to achieve functional integrity and survival of T-cell subsets in activating environments remains poorly understood. My talk will review aspects of Notch1 receptor-mediated signaling in T-regulatory cells, a cell-type in the mammalian immune system, activated in response to inflammatory challenges. I will discuss experiments, which show that the integration of autophagy and Notch1-signaling is important for dynamic changes in mitochondrial function and T-regulatory cell survival. Evidence suggesting a role for Notch1 signaling in lineage stability of T-regulatory cells, will also be presented.

Blood relatives: The role of OCIA domain proteins in stem cell maintenance

Maneesha Inamdar
Jawaharlal Nehru Centre for Advanced Scientific Research, Bengaluru
inamdar@jncasr.ac.in

Tissue homeostasis is orchestrated by the interplay of external stimuli and intracellular regulatory programs. The blood vascular system is particularly sensitive to stressors and perturbations can often lead to disease. Molecular control of self-renewal and differentiation is key to maintaining blood cell homeostasis. Despite the enormous clinical potential of hematopoietic stem cells, mechanisms that regulate their vital properties of self-renewal and multi-potency are not completely understood. Using various model systems we identified a network of interactors that regulate HSCs, mediated by the OCIAD family of proteins. Our findings reveal additional controls that may modulate key tumor suppressors to influence stem cell fate in haematopoiesis.

Systemic control of myeloid cell differentiation and cellular immune response

Tina Mukherjee
Regulation of Cell Fate, inStem
tinam@instem.res.in

Drosophila hematopoiesis in addition to near neighbor interactions is influenced by systemic cues of sensory olfactory origin. While underlying mechanisms mediating this cross talk are being investigated, the physiological basis to the olfaction-immune axis remains unclear.

The olfactory system is a unique sensory modality that promotes animal survival by detecting odors to discriminate between favorable and unfavorable conditions by initiating appropriate behavioral changes. Our work demonstrates that sensing environmental odors during development is necessary for priming immune precursor cells to respond effectively to infections. An unexpected finding is the extent to which environmental odors can influence immune competency. The talk will dwell into some of these recent observations and elucidate the importance of odor perception in innate cellular immunity by invoking the use of GABA as a metabolite.

Neuroprotection through targeting macroglia?

Siddharthan Chandran
University of Edinburgh
siddharthan.chandran@ed.ac.uk

Parsing the contribution of fibroblast heterogeneity to tissue fibrosis

Colin Jamora
Centre for Inflammation & Tissue Homeostasis, IFOM-inStem Joint Research Laboratory, inStem
colinj@instem.res.in

Fibrosis is a pathological derivative of the wound-scarring process in which excessive deposition of extracellular matrix leads to tissue hardening and loss of organ function. A central process in both scenarios is an “activated fibroblast”, an umbrella term that includes seemingly contradictory processes such as increased migratory and contractile properties. This raises an interesting possibility that subpopulations of fibroblasts are specialized for specific functions and are under distinct regulatory controls. Using a mouse model of skin fibrosis, we have found that the matricellular protein Mindin specifically causes migration of the reticular subpopulation of dermal fibroblasts, while the papillary fibroblast are the cells that exhibit a contractile phenotype. Furthermore, we found that Mindin stimulates migration in reticular fibroblasts via Fyn kinase activity while contraction in the papillary dermal fibroblasts is mediated by c-Src. Overall this work is unravelling how fibroblast heterogeneity contributes to different aspects of fibrogenesis.

Airway progenitors for alveolar repair: Opportunities for many?

Arjun Guha
Regulation of Cell Fate, inStem
arjung@instem.res.in

Alveolar epithelial cell damage caused by exposure to environmental toxicants and infectious agents will, unless repaired, compromise respiratory function. Efforts are underway to delineate the stem/progenitor cells that contribute toward alveolar repair. Studies in the mouse model have implicated specific alveolar and airway epithelial cells and I will present our work on Uroplakin 3a+ (Upk3a) airway Club cells in this context. Lineage analysis of Upk3a+ cells shows that they are airway progenitors, that they do not contribute to the maintenance of the alveolar epithelium in the uninjured lung long-term and that they do contribute toward alveolar post-injury repair. Upk3a+ Club cells can be distinguished by marker expression from lineage-uncommitted airway progenitors (Krt5+ cells, BASCs) that are also thought to contribute. Thissuggests that both lineage-committed (Upk3a+ Club cells) and lineage-uncommitted (Krt5+, BASCs) airway progenitors participate in the repair of the alveolar epithelium.

Sarcomeric disease due to non-sarcomeric proteins

Dhandapany Perundurai
Centre for Cardiovascular Biology and Disease, inStem
dhan@instem.res.in

Hypertrophic cardiomyopathy leading to thickening of heart muscle affects one in every 250 persons. Various sarcomeric gene mutations have been shown to cause this condition. However, in a significant number of cases, the cause remains unknown, especially in patients who are negative for sarcomeric gene mutations. We use South Asian-specific next-generation sequencing data, cellular models, and mouse genetics to explore novel genes and their functions related to cardiac hypertrophy. Our results revealed surprising roles for several non-sarcomeric proteins including receptors in cardiac hypertrophy. My talk will focus on newly identified receptor proteins and their broad implications in cardiomyopathy.

C9ORF72 repeat expansion causes vulnerability of motor neurons to Ca^{2+}-permeable AMPA receptor-mediated excitotoxicity

David Wyllie
University of Edinburgh
David.J.A.Wyllie@ed.ac.uk

Repeat expansion of a GGGGCC intronichexanucleotide in the C9ORF72 gene is the most common cause of familial amyotrophic lateral sclerosis (ALS) and accounts for ~10% of sporadic ALS cases. Nevertheless, how such mutations result in selective motor neuron loss remains unclear. Moreover, recognition that familial and sporadic ALS are largely phenotypically indistinguishable and share pivotal pathological features, highlights the value of the study of familial forms of ALS to provide insight into ALS mechanisms. Several studies have suggested that one consequence of C9ORF72 mutations is aberrant glutamatergic signalling mediated by α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs). Recently we have employed a combination of RNAseq and electrophysiological analysis on control and patient iPSC-derived motor neurons which demonstrates that the C9ORF72 mutation causes increased expression of Ca^{2+}-permeable AMPARs that leads to an increased vulnerability to excitotoxicity. Importantly these deficits are abolished by CRISPR/Cas9-mediated correction of the C9ORF72 repeat expansion, thus, establishing causality between mutation and phenotype on an isogenic background.

Large scale genomic analysis of pedigrees: A first year perspective

Mahendra Rao
Centre for Brain Development and Repair, inStem
rao1789@gmail.com

Whole genome and exome sequencing strategies have proven invaluable in generating potential target that may be relevant in the etio-pathology of disease. The bioinformatics pipeline for such an analysis is still being developed for polygeneic disorders and two parallel approaches have been attempted. A genome wide association type analysis, which looks at relatively segregated populations using a large N to extract signal from noise. An alternative approach is based on examining well characterized families with a defined disorder and performing a pedigree analysis as has been done successfully for monogenic disorders. Both approaches have their limitations. In our collaborative multi institute project we have chosen to evaluate the pedigree based approach and to overcome some of its limitations by developing functional assays using iPSC and gene editing technologies. We will present our efforts on developing the pipeline, enrolling families and performing the initial analysis. Our initial results suggest that it is important to develop control and reference data sets to reduce the number of leads and that choosing the correct familial controls may be important in reducing the number of leads. We have also evaluated functional readouts that may be used in a quick screen that is scalable and reliable and suggest that calcium imaging coupled with neurite outgrowth and simple measures of neuronal health may be sufficient for an initial screen.

How does the same receptor in the amygdala give rise to both fear and anxiety?

Sumantra Chattarji
Centre for Brain Development and Repair, inStem
shona@ncbs.res.in

Although antagonists of the group I metabotropic glutamate receptor subtype mGluR5 prevent fear and anxiety, little is known about how the same receptor in the amygdala gives rise to both. Combining *in vitro* and *in vivo* activation of mGluR5, we identify specific changes in intrinsic excitability and synaptic plasticity in lateral amygdala neurons that give rise to temporally distinct and mutually exclusive effects on fear-related behaviors. The immediate impact of the activation of mGluR5 is to produce indiscriminate fear of both tone and context. Surprisingly, this state does not interfere with the proper encoding of tone-shock associations in a discriminative fear conditioning paradigm. These results provide a new framework across biological scales for examining mGluR-plasticity in the amygdala, and its behavioral consequences for emotional function in neurodevelopmental disorders like Fragile X Syndrome.

FMRP and miRISC modulate the synaptic protein synthesis

Ravi Muddashetty
Centre for Brain Development and Repair, inStem
ravism@instem.res.in

To understand the role of FMRP during neuronal differentiation and development we investigated its function beyond the realm of "mGluR theory". This quest has taken us from synapse to nucleus to nucleolus and back. But in the current talk I will focus on our work on the role of FMRP along with miRISC (microRNA mediated silencing complex) in regulating protein synthesis in response to NMDAR stimulation. Using ribosome profiling we show a distinct translation response to NMDAR and mGluR stimulation and demonstrate reversibility of miRISC mediated inhibition as a common mechanism to regulate translation downstream of both receptors. Interestingly we have found that the dynamic interaction of FMRP-MOV10-AGO2 determines translation in response to NMDAR and distinguishes it from mGluR stimulation. We have identified that phosphorylation status of FMRP acts as a molecular switch downstream of both receptors but in an inverse mannerthus indicating its versatile role at synapse.

Deconstructing the synaptic and cellular basis of neuronal excitability in a rodentmodel of Fragile X Syndrome

Peter Kind
University of Edinburgh
P.Kind@ed.ac.uk

Early intervention in cancer through the tumour suppressive mechanisms that maintain genome stability

Ashok Venkitaraman
Medical Research Council (MRC) Cancer Unit, University of Cambridge,
arv22@hutchison-mrc.cam.ac.uk

Inactivation of the tumour suppressive mechanisms that control genome stability accompanies the transition from pre-malignant to invasive stages of cancer in epithelial tissues. I will discuss insights emerging from our lab's work concerning the cellular and molecular organization of these tumour suppressive mechanisms, and new approaches that offer the potential for early intervention through improvements in detection, risk stratification or therapy.

Deciphering ligand induced conformational changes in the Sodium Galactose Transporter (SGLT)

Jeff Abramson
University of California, Los Angeles, USA
jabramson@mednet.ucla.edu

Secondary active transporters use ionic gradients to pump specific molecules across the otherwise impermeable membrane bilayer that surrounds all cells and organelles. These proteins are essential components for cell communication, function and survival and are important targets for drug development. The transport of substrates against a transmembrane concentration gradient proceeds through a series of discrete conformational transitions, during which the protein's substrate-binding site is accessible only to one side of the membrane at any given moment. In this work, we engineered a large number of double cysteine mutants—based on our crystal structures—and performed double electron-electron resonance (DEER) to gain insights into the dynamics of ligand-induced motions in SGLT.

Molecular explanations of why some Walleyes turn blue

Ramaswamy S.
Technologies for the Advancement of Science, inStem
ramas@instem.res.in

Sandercyanin, discovered in the skin mucus of the North American walleye. We observed that apo-Sandercyanin predominantly exists as a small non-fluorescent colorless monomer in nature but quickly oligomerizes to a blue colored homo-tetramer of 75 kDa on binding of Biliverdin (BLA). Spectroscopic properties BLA-bound Sandercyanin shows strong far red fluorescence maxima at 675nm, when excited at 375nm or 630nm, with minimal overlap with the excitation spectra in the blue region. Fluorescence intensity of Sandercyanin in walleye mucus does not decrease on long exposure to UV or red light. Crystal structures show that Sandercyanin is a tightly packed tetramer with each monomer non-covalently bound to one BLA. Our work provided a molecular explanation of adaptation observed in nature to environmental pollution. In an attempt to develop Sandercyanin as a Fluorescent protein tag using a structure based engineering, we now have monomeric form of Sandercyanin that shows similar fluorescence properties as the wild type.

Sensing across scales: Fluorescent biosensors for cellular dynamics and discoveries in natural light sensing

Akash Gulyani
Technologies for the Advancement of Science, inStem
akashg@instem.res.in

Cell signaling is complex and signaling proteins in living cells are tightly regulated in space and time. This allows the same protein or sets of proteins to perform multiple and sometime opposing functions. Fluorescent biosensors that can help visualize protein activation patterns in living cells are valuable for understanding this complexity. I will talk about how we have designed a new class of fluorescent sensors with diverse readouts, while also discussing new biological insights derived in the area of cell migration and cell fate changes. The talk would highlight the first fluorescent biosensor that can specifically visualize the activity of a single kinase (Fyn) within a whole family of kinases (Src family kinases). Fyn is a critical signaling node in multiple cellular contexts, and some specific signaling examples will be discussed. I will also briefly showcase our efforts in the area of natural light sensing. I will specifically highlight how flatworms can perform complex light sensing and processing with very 'simple' light sensing apparatus, while also possessing multiple light sensing modes.

Interrupting intracellular signaling via the molecular recognition of phosphorylated substrates by the BRCT domains

Kavitha Bharatham & Gayathri Sadasivam
Centre for Chemical Biology and Therapeutics, inStem
kavithab@instem.res.in&gayathrisadasivam@instem.res.in

Intracellular signaling pathways emanate from enzymes like protein kinases that conjugate post-translational modifications (PTMs) to their substrates, whose molecular recognition by specific protein domains underlies signal propagation. The interruption of PTM recognition using selective chemical tools is potentially an attractive approach for the modulation of intracellular signaling, but its structural and chemical foundations remain poorly understood. Here, we will report our efforts to discover and develop small-molecule chemical tools that interrupt the molecular recognition of phosphorylated substrates by the BRCT domains, a widespread family of protein folds implicated in the pathways that maintain genome integrity from bacteria to humans.

Nanoengineering for discovery and disease mechanism related to cytoskeleton

MinhajuddinSirajuddin
Centre for Cardiovascular Biology and Disease, inStem
minhaj@instem.res.in

Eukaryotic biological motions across scales and orders of magnitude involve cytoskeleton elements. Because of their importance in cell division, motility and muscle contraction, mutations in cytoskeleton are frequently associated with human pathology. Our lab is focused on understanding how cytoskeleton assemblies coordinate during physiological and their deregulation during disease conditions. In this talk I will highlight work from our lab, which utilizes the power of nanoengineering and *in vitro* reconstitution to uncover new findings in cytoskeleton biology, and how we can bridge the knowledge gap between clinical findings and molecular mechanism.

Engineering of biomaterials enables 'disease-responsive drug delivery' for the treatment of inflammatory bowel diseases

Praveen Kumar Vemula
Technologies for the Advancement of Science, inStem
praveenv@instem.res.in

Design of disease-specific drug delivery vehicles is bringing a new dimension to biomaterial engineering for medical applications.In this approach, identifying a biomarker such as an enzyme, cytokine, protein or antibody that is specific for a disease of interest is key. Additionally, one needs to design a molecule or drug-loaded biomaterial, which is stable at normal physiological conditions to serve as a drug depot. Importantly, this biomaterial should be able to degrade/disassemble in response to the disease-specific biomarker to release the drug. This approach is giving a new direction to the design of drug delivery vehicles to impact disease management in a multifactorial manner.Subsequently, it will lead to establishing a novel chemistry to generate molecules/materials that can be degraded/dissociated in response to the identified biomarker. In this talk, I will discuss our recent work which enabled us to find an active gut metabolite to alleviate inflammatory bowel diseases.

Gene therapy for hemophilia B in India

Alok Srivastava
Centre for Stem Cell Research (CSCR), CMC Vellore
aloks@cmcvellore.ac.in