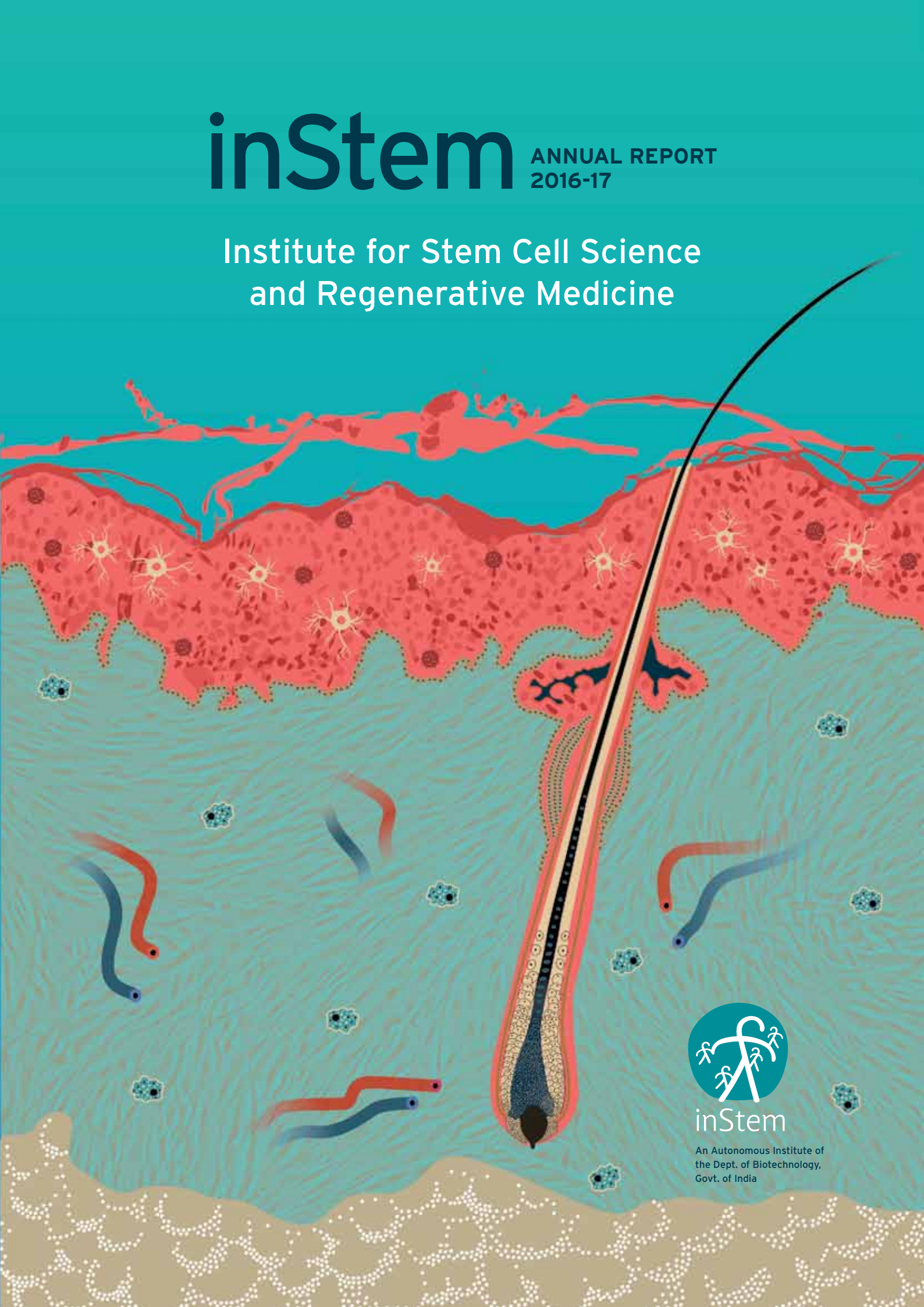
inStem ANNUAL REPORT 2016-17

Institute for Stem Cell Science and Regenerative Medicine



inStem

An Autonomous Institute of the Dept. of Biotechnology, Govt. of India

inStem new building

inStem

ANNUAL REPORT 2016-17

Institute for Stem Cell Science
and Regenerative Medicine

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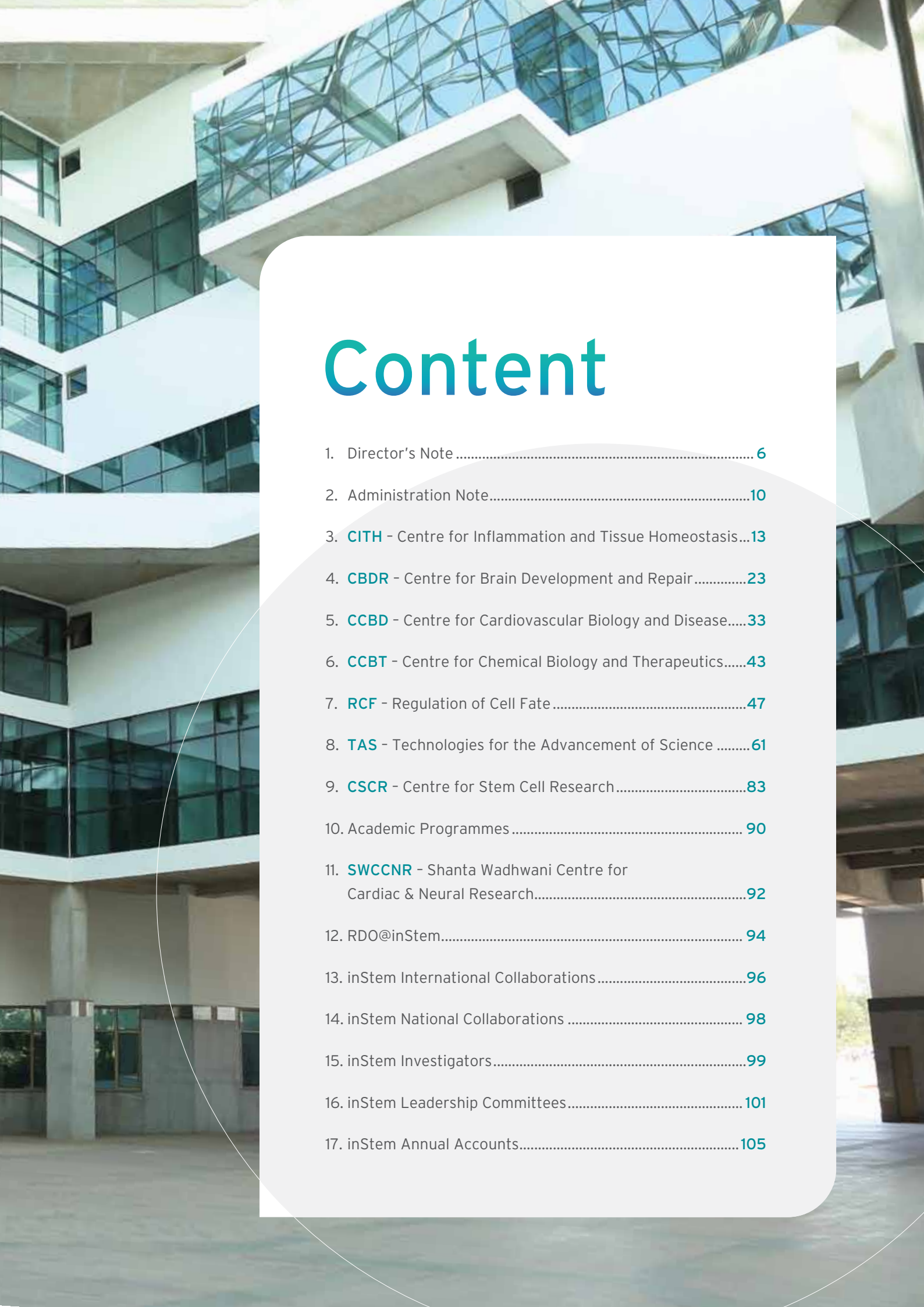
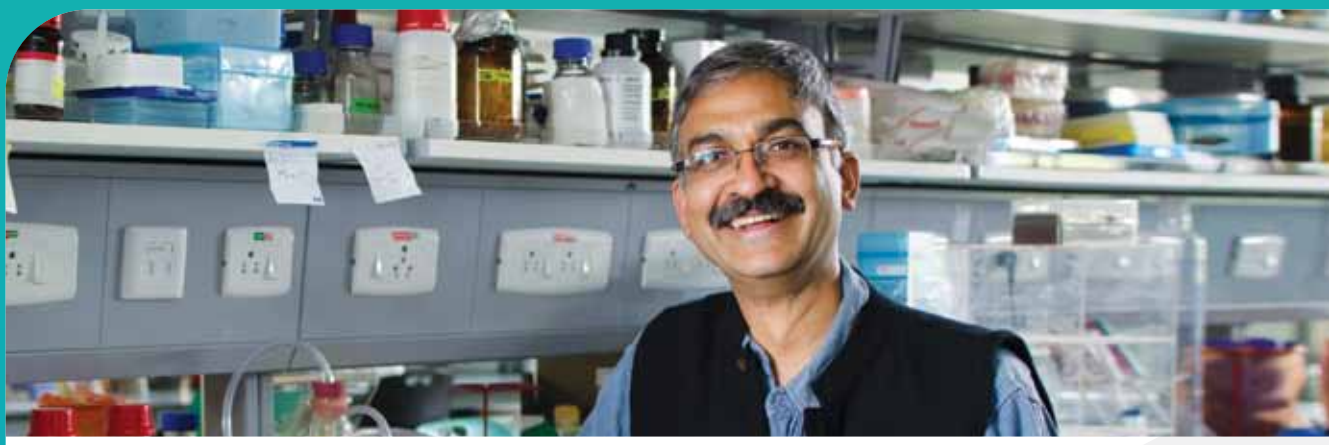


Content



Director's Note

With the approval of inStem's final project budget before we transition into an institutional mode, our move into the new building becomes a reality. Designed by Kanvinde and Rai Architects, and constructed by JMC and URC Construction Agencies, the new inStem laboratory complex is built to accommodate theme-centric research activities. Here, we hope the culture of 'collaborative inquiry' continues to grow and scale new heights in both basic and translational research. We anticipate our move into the new laboratory complex will be complete before the year 2017 is out.

While the new building has been in gestation, Science at inStem has been slowly maturing, and this Annual Report provides a good account of the same, so I do not dwell here on any specifics. I must appreciate the efforts of our Dean, Apurva Sarin, and the entire Scientific Advisory Board for providing encouragement necessary for our metamorphosing from a crew of loosely knitted individuals to a vigorously collaborative set of themes. We now need to start giving serious thought to the direction and coherence of the thematics that are emerging at inStem. Our SAB has something to say about this 'It is likely that new opportunities will arise, but thought should be given to how such new developments can be integrated to benefit inStem, and thought should be given to potential exit plans'.

One mechanism that inStem has at its core is the fact that most of the themes receive generous support from extra mural funding sources via GoI agencies DBT and DST, as well as an increasing number of private donors. This has created a very high level of expectation and realization of possibilities at inStem. The continued existence of these thematics will strongly depend on their

ability to attract globally competitive funding to scale and create new opportunities for expanding their mandates.

An example of such a trajectory is the Centre for Chemical Biology and Theraputic (CCBT) theme. This has matured in its outlook from an extremely focused thematic, exploring the 'druggability' of specific cancer targets that are based on the 'un-druggable'. By sheer perseverance and clear thinking by the CCBT team, and outstanding leadership from Prof Ashok Venkitaraman, CCBT has provided extremely convincing evidence that new cancer targets and corresponding small molecule drugs are foreseeable in near future. This effort is now opening itself up to entertain a number of engagements on the campus and beyond, in different protein-protein interaction based disease targets, where such a bold approach may open up new possibilities for Chemical Biology. This is an excellent example of building up technologies and capabilities within a thematic, which is opening up ways to ask new questions in therapeutic research in general. We wish this theme all success in its efforts in building up the next phase of its activities.

Another theme that enjoys extramural funding is the Centre for Brain Development and Repair where two major funded programmes are joined at the hip, The Centre for Neurodevelopmental Synaptopathies (CNS) and the Accelerator program for Discovery in Brain disorders using Stem cells (ADBS). CNS led by Shona Chattarji at inStem and jointly run by Siddarthan Chandran and Peter Kind from Edinburgh, with vital catalytic support from the Wadhwani Foundation, has made excellent progress. The inclusion of the ambitious tripartite collaborative initiative, ADBS, led by Collaborative Science Chair, Mahendra Rao at inStem with NCBS (Raghu Padinjat) and NIMHANS (Sanjeev Jain) under this umbrella has provided unprecedented opportunities to develop synergies. The clinical links that the CNS programme has been mandated, become available via the ADBS program, and in parallel, the capabilities that the CNS programme has put in place are tremendous opportunities for the ADBS programme. These two flagship programmes of the DBT in the area of brain disorders, its understanding, and its remediation by the deployment of Stem cell technology, are being pioneered at inStem. A Biorepository of Stem Cell lines from patients is already under way and should provide a tremendous resource for the scientific community, a portent of very exciting times ahead.

InStem has inducted a new theme that is jointly supported by the Indian subsidiary of the Tata Institute of Genetics and Society (TIGS) at University of California, San Diego, USA, TIGS-India. The Centre for Active Genetics, called TIGS-Centre at inStem (TIGS-CI), shall focus on the broad applications of active genetics, an efficient gene editing technology, pioneered by TIGS researchers in UC San Diego, for beneficial and ethical societal impact in the fields of health and agriculture. The deployment of the technology of active genetics in stem cell and almost any model organism is an extraordinary opportunity for researchers at inStem and elsewhere on the campus to grasp, while TIGS-CI is dedicated towards clear translational goals in the area of control of insect vectors of infectious agents. We are particularly happy to welcome the global head of TIGS and first Scientific Coordinator of TIGS-CI, Prof Suresh Subramani (former Executive Vice Chancellor for Academic Affairs and a Distinguished Professor of Molecular Biology at the UCSD) and long time friend of our campus to lead this effort as a Collaborative Science Chair.

At the level of the cluster, NCBS and inStem continue to develop close linkages at the collegial and institutional level. This is most apparent in the setting up of joint facilities, such as the ambitions Structural Biology initiative, propelled by S Ramaswamy at inStem and Upi Bhalla and Vinothkumar from NCBS in the form of a National Facility for Cryo-EM and a Big Data facility. We are extremely proud to announce the successful installation (as this report goes to print) of India's first Cryo-EM. We thank the DBT for providing timely funds for this initiative of the Cluster at Bangalore.

Over the last year our recently appointed Head of Administration, Mr Ramachandran, was lured back to his old job, as the Deputy Commissioner of the Navodaya Schools Program in Delhi. We wish him well, and thank him not only for his indelible mark of administrative acumen, but also stewarding our efforts in obtaining a coveted tax-exempt status. This is necessary for building up our donations and endowments, critical for our growth and flexibility in functioning. We are grateful to Mr Krishnama Raju (former Head of Administration of the Raman Research Institute) for stepping into the breech and providing us his knowledge and time, to tide things over.

As a consequence of a recently concluded Indian Philanthropist Initiative held on our campus, both inStem and NCBS were granted a substantial sum to support a Science without Borders Initiative (bringing international investigators to our campus, providing travel opportunities for our young researchers to travel to workshops, carry out collaborations in places much beyond our shores, and providing support for outstanding international fellows). For this, on behalf of all of us at this campus, I would like to offer our sincere appreciation to Mr T T Jagannathan, Chairman of TTK Prestige for his generous donation. I would like to thank Kris Gopalakrishnan and Kiran Mazumdar for co-sponsoring the IPI event that facilitated this, and for remaining fully engaged with our campus, and being a constant source of support and inspiration.

While each theme has developed a distinct identity and a modus operandi, it would be important for each of the Centres to reflect on their future growth, and ensure that the sum of their parts adds up to more. As we move to the 10 year mark at inStem, a time to reflect on these efforts is well nigh. Here again, comments from our SAB ring a loud bell: 'With the imminent prospects of moving inStem to the new building, it is important to make careful assessment of how inStem has operated over the past 10 years, with respect to both positive and negative impact of some of the past decisions'. And include pointers about developing into a mature institute such as 'at this important

juncture in the development of inStem as a full-fledged institution that there is a need to examine its broader goals and to initiate discussions aimed at defining a consistent set of best practices written down in the form of bylaws and made transparent to the community'. There is clearly much work cut out here.

While we do have a few more hurdles to cross before we are a well staffed, funded and run, inStem is developing into an institute in its own right. I feel that the future is bright, so do bring your sunglasses with you for our journey through 2018.

S MAYOR
Director, inStem

2

Administration Report

The Institute has completed its eighth year in its pursuit for excellence in stem cell research and allied areas. Following the approval of the Revised Cost Estimate (RCE) by the Department of Biotechnology, Government of India, the infrastructure development has resumed and the work is in full-swing. Every effort is being made to ensure that these works will be completed by the end of March 2018.

The National Centre for Biological Sciences (NCBS)/Tata Institute of Fundamental Research and the Centre for Cellular and Molecular Platforms (C-CAMP) continued to extend shared services to inStem as participants in the Bangalore Life Science Cluster (BLiSc).This has resulted in sharing of resources at optimum level as well as saving in costs, if these services were to be run independently.Based on the positive experience and economies that are being derived a formal system is being proposed through a memorandum of understanding between the participating institutions.

CSCR's (a center of the Institute situated at Vellore) accounts are integrated into the accounts of the Institute for the year.

The table below indicates the status of grants received and the manpower count at the end of 31.3.2017.

DETAILS	2015-16	2016-17
Core grants received	₹ 384.7 million	₹ 687.00 million
EMG grants received	₹ 502.2 million	₹ 908.87 million
No. of active grants	50	55
Manpower	215	232

Within the limited positions that were available to the Institute and with the support of temporary staffchecks and balances were introduced wherever feasible to ensure continued systemic improvements as well as to provide services to the scientific and technical activities as smoothly as possible. In achieving this, colleagues in Scientific, Technical (including in services and construction) groups worked in unison with the administration, and its appreciation for their support is much appreciated.

With the approval of RCE and the sustained efforts of the authorities of the Institute and those in the Department of Biotechnology to obtain pendingapproval for positions combined with the availability of its own building, the Institute functioning will be much smoother and its contributions will increase considerably in the years to come.

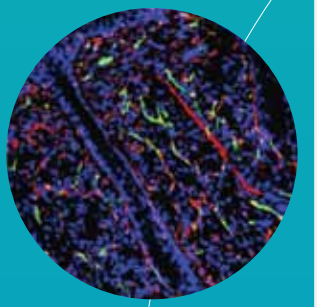
A N Ramachandran (until 28th January 2017)
K Krishnama Raju (from 29th January 2017)
Head, Administration, inStem

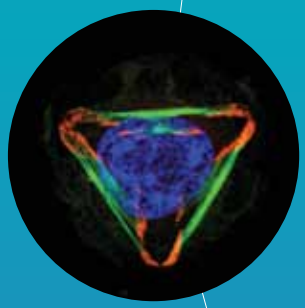
inStem new building

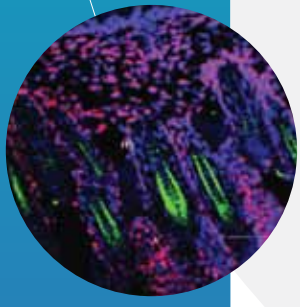
3

CITH

CENTRE FOR INFLAMMATION AND TISSUE HOMEOSTASIS







The aim of the Centre for Inflammation and Tissue Homeostasis (CITH) is to generate the knowledge and tools that would help make the enormous promise of regenerative medicine a reality. The promise of regenerative medicine lies in the potential to replace or repair tissues lost through aging, disease, or injury. Major research programs in CITH are designed to elucidate the molecular mechanisms of wound healing, cell and tissue aging, the role of small RNAs in regulating stem cell behavior and how cells perceive and react to its physical environment. These studies span the full gamut of experimental systems, from chemical modifications of DNA and proteins, to macromolecular protein assemblies, to the biophysical characterizations of cells and tissues. Though these research programs are rooted in basic biology, our work has involved the investigation of common diseases such as diabetes, fibrosis and cancer where many of these same processes are deregulated and help drive disease progression. Using the skin as a model system, over the past year CITH laboratories have made important advances in understanding new roles for immune cells, mechanical signals, and small RNAs in regulating stem cell behavior, and have deciphered the molecular and cellular basis of skin aging.

COLIN JAMORA
Theme Coordinator

3.1

Colin Jamora

colinj@instem.res.in

MOLECULAR MECHANISMS OF WOUND-HEALING AND DISEASES WITH A "WOUND SIGNATURE"

THE IFOM-INSTEM JOINT RESEARCH LABORATORY WORKS ON DECIPHERING THE MOLECULAR MECHANISMS UNDERLYING WOUND HEALING. THE GOAL IS TO UTILIZE THIS KNOWLEDGE TO DEVELOP THERAPIES FOR DISEASES WHERE WOUND HEALING IS DEREGULATED, SUCH AS IN DIABETES, FIBROTIC DISEASES AND CANCER.

The Jamora lab operates under the auspices of a research agreement between the FIRC Institute for Molecular Oncology (IFOM) in Milan, Italy and inStem resulting in the IFOM-inStem Joint Research Laboratory. Our research is focused on understanding the mechanisms underlying wound healing. The process of wound healing is often found to be impaired in many systemic diseases. In diabetic patients, for instance, tissue loss is observed due to the inability to heal, whereas in patients suffering from fibrosis of the skin, liver or kidney, excessive scar tissue formation compromises organ function. Moreover, tumor progression reproduces many hallmarks of the wound microenvironment and thus our program has seamless connections with IFOM, an institute that focuses on the molecular mechanisms underlying tumor formation and development. Our goal is to understand the molecular and cellular crosstalk that underlies the restoration of tissue integrity in normal wound healing

and how it is perturbed in pathological conditions, thereby revealing potential avenues of therapeutics development for a host of common diseases.

Various projects are underway to understand the extensive intercellular communication and signaling pathways that mediate the inflammatory, proliferative and remodeling phases of the wound healing response. These studies are being conducted in collaboration with biologists and engineers from within India and internationally, with clinicians from Christian Medical College (CMC) Vellore, India as well as with prominent entities in India's biotech industry such as Hindustan Unilever, L'Oreal Inc. and Biocon Ltd. Together with these collaborators, we can address our experimental objectives with a multidisciplinary approach with the goal of understanding the basic biology of wound healing and developing treatments that can be used in the clinic when these wound-healing pathways go awry and lead to disease.

Two recent advances in our work with fibrosis will be highlighted to provide a flavor of the ongoing projects in the IFOM-inStem Joint Research Laboratory. The need to understand the basic processes leading to tissue fibrosis is borne out by the fact that it contributes to 1/3 of all deaths worldwide yet there are no effective treatments for this disease:

1. The crosstalk between immune cells and dermal fibroblasts in the skin that promotes fibrosis development.

Fibrosis is the pathology that arises when the scarring process of the wound healing response does not stop within the normal time frame. This is a very common occurrence and affects almost all tissues in the body leading to diseases such as cirrhosis of the liver, pulmonary fibrosis in the lung, atrial fibrosis in the heart and keloids in the skin. Fibrosis is a complex process, but a common characteristic in all cases is a strong inflammatory response. How inflammatory cells are recruited to the tissue and how they contribute to this over-scarring is unknown. We have found that a protein known as Plasminogen Activator Inhibitor-1 (PAI-1), that is normally associated with blood clotting, is capable of recruiting a specialized type of immune cell called a mast cell into the fibrotic tissue. In addition, PAI-1 mediates the interaction between these mast cells and the resident dermal fibroblasts of the skin to induce these fibroblasts to produce more of the scar material. Consequently, we have identified a new process mediated by PAI-1 that has the potential of limiting the amount of scars that form and thereby delay or prevent the onset of fibrosis.

2. Identification of a novel protein that activates dermal fibroblasts in the skin fibrotic disease scleroderma

The components that comprise a scar are produced and released from the dermal

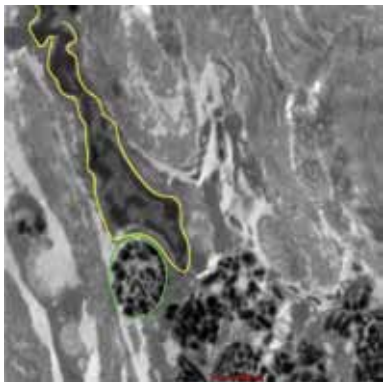


Figure 1: Adhesion of mast cell (outlined in green) and dermal fibroblast (outlined in yellow) in the skin of patients with the fibrotic skin disease scleroderma

fibroblasts in the skin. Therefore, one strategy of treating fibrosis would be to control the output of scar proteins from these fibroblasts. However, the signals that control scar protein production in these cells are unknown. We have recently established that the protein known as Mindin is capable of activating the pro-scarring activity of the fibroblasts. Importantly, when we remove this protein in a mouse model prone to the development of skin fibrosis, this overscarring phenomenon is prevented. Analysis of skin samples from Indian patients with the fibrotic skin disease known as scleroderma also shows an elevated level of Mindin, consistent with this protein playing a similar role in the human disease as it does in our mouse model of fibrosis.

Overall, we have identified two different proteins that can be targets of therapeutics thereby potentially alleviating the development of fibrosis. This would fill a major clinical need as there are currently no effective treatments for this disease.

INVITED TALKS

1. Regulation of Epithelial Stem Cells During Wound Healing. Indian Institute of Toxicology Research, Lucknow, India 2017.

2. Regulation of Epithelial Stem Cells During Wound Healing. DBT-NER Workshop in Stem Cell Biology: Advanced Centre for Treatment, Research & Education in Cancer (ACTREC), Mumbai, India 2017.

3. Regulation of Epithelial Stem Cells During Wound Healing. Stem Cell and Regenerative Medicine Workshop: Anna University, Chennai, India, 2016.

4. Mechanical and Epigenetic Regulation of Wound Healing. Mechanical Forces in Cell Biology Conference: National Centre for Biological Sciences, Bangalore 2016.

5. Epigenetic Regulation of Wound Healing IFOM-inStem Conference on Inflammation and Tissue Homeostasis, Bangalore 2016.

6. Best practices in PhD supervision. Manipal University Research Colloquium: Manipal University, India 2016.

7. Elucidating the signals that orchestrate tissuerepair and promote disease. Centre for Biosystems Science and Engineering Annual Symposium:Indian Institute of Science, Bangalore 2016.

3.2

Shravanti Rampalli Deshpande

shravantird@instem.res.in

HISTONE METHYLTRANSFERASES GUIDING DEVELOPMENT AND AGING

BROAD AREAS OF RESEARCH INTEREST IN THE LABORATORY LIE AT THE INTERFACE OF EPIGENETICS AND STEM CELL BIOLOGY. WE ARE ADDRESSING THE ROLE OF REPRESSIVE HISTONE METHYLTRANSFERASES IN THE COMMITMENT OF CELL FATE FROM STEM CELL THROUGH AGING.

Lysine methyltransferases (KMTs) that deposit methylation marks on proteins are critical determinants in various facets of development and disease. Deletions mutations of KMTs lead to either embryonic lethality or developmental defects. In addition, mis-regulation of KMTs is often noticed in multiple cancers. Till date, KMTs have been best studied for their role in methylating histones thereby regulating gene expression. Owing to the scarcity of knowledge on non-histone methylations, it remains elusive how the non-histone methyl proteome impacts development and disease.

At inStem, my laboratory is investigating the canonical and non-canonical mechanisms by which KMTs regulate cell plasticity in development and how perturbations of these mechanisms lead to disease states. Currently, we are studying two KMTs namely EZH2 and

EHMT1 that are critical for development. In the past few years our quest to identify the non-histone methylations performed by EZH2 and EHMT1, not only provided information about development and disease but also lead to the discovery of a novel role of EHMT1 in aging. In addition, we have been successful in generating human stem cell based tools and mouse models to ask the questions as to how histone (canonical) and non-histone proteins methylations (non-canonical) could converge to ensure desired biological outcomes.

Following key programmes are being studied at my laboratory:
(i) Mechanisms underlying cellular plasticity regulated by KMTs
(ii) Contribution of KMTs to aging and age related diseases
(iii) Function of KMTs in tissue repair and homeostasis

Mechanisms underlying cellular plasticity regulated by KMTs

Cellular plasticity is characterized by acquisition of new identity towards alternative fate and function. Such plasticity is an essential component of normal physiology and disease progression. The success of enforced cell conversion in culture dish that can be tailored to make patient specific cells for cell replacement therapy is a major area of interest. At the molecular level, cell state conversion must overcome several barriers such as the existing epigenetic landscape, transcription factor expression and protein-protein interactions in response to stimuli. While large efforts have been put forth to identify factors that influence cellular plasticity, a comprehensive understanding of this intriguing process still remains elusive.

To get a complete overview of the cellular plasticity and its consequence on biological outcomes, we are studying KMT mediated regulation of histone and non-histone methylation during cellular reprogramming.

Function of KMTs in tissue repair and homeostasis

In vivo cell type conversions are commonly seen in lower organism to build lost tissue, however this process is much less recognized in mammals. Recently, there is a surge in the literature demonstrating such conversions that are triggered by injury as repair response to promote tissue homeostasis. A compelling example of such adaptive transdifferentiation occurs in skin injury wherein TGF-B released upon wounding converts fibroblasts to myofibroblasts. The process of tissue repair is a highly complex event and requires orchestrated and coordinated control on transient activation and repression of genes from cells of different origin. While histone methyltransferases (HMTs) are key players of development and disease, their role in wound healing still remains poorly defined. Studies in our laboratory indicated overexpression of EZH2 during the process of normal wound healing. Next, we are investigating the effects of EZH2 in differenatiation/transdifferentaition of skin cells during repair and aging.

Contribution of KMTs to aging and age related diseases

Aging is a pleiotropic and time dependent process governed by genetic and environmental factors resulting in loss of normal cellular plasticity. It is the single most undisputable risk factor for several prevalent diseases including, neurodegenerative, metabolic, cardiovascular disease and cancer. Thus, from both basic science and translational perspectives, intense effort has been dedicated to uncover the molecular mechanisms underlying cellular aging. At molecular level, aging leads to degenerative epigenomic changes in somatic as well as stem cell components that are responsible for the progressive loss of homeostatic and regenerative potential of a given tissue.

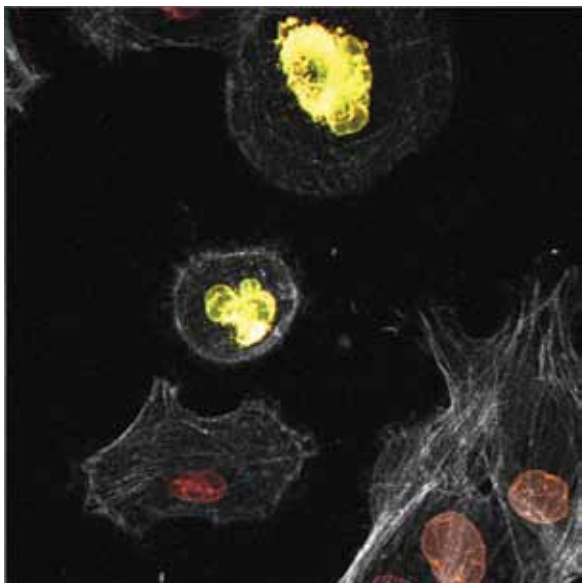


Figure 1: Nuclear defects in LaminB1 methylation deficient mutant cells.

Traditionally the function of HMTs has been attributed to altering the epigenome structure by methylating histone tails during aging. In my laboratory, we focused our studies on non-histone methylation of HMTs that contributes to loss to cell plasticity thereby resulting in aging. Towards this, we have demonstrated that the Euchromatic histone methyltransferase mediated histone lysine 9 methylation and non-histone methylation of LaminB1 are critical determinants of heterochromatin organization and its impact on fundamental changes associated with aging process.

We are also investigating the contribution of EHMT1 in age related metabolic disease such as diabetes and obesity. Our data indicates deregulation of EHMT1 expression can serve as early biomarker for the pathogenesis of obesity.

INVITED TALKS

1. Epigenetic regulation of somatic cell plasticity. IFOM-inStem Conference on Inflammation and Tissue Homeostasis. Bangalore. February 3rd-5th, 2016.

2. Specification of peripheral heterochromatin organization via non-histone interaction of Ehmt1. 11th Asain Epigenomics Meeting. Bangalore. September 30th, 2016.

3.3

Srikala Raghavan

srikala@instem.res.in

EPITHELIAL HOMEOSTASIS AND INFLAMMATION: INTEGRINS AND SMALL RNAs

RESEARCH IN THE RAGHAVAN LAB FOCUSES ON UNDERSTANDING THE ROLE OF INTEGRINS, ITS ASSOCIATED PROTEINS AND SMALL RNAs IN MAINTAINING THE STEM CELL NICHE AND EXTRACELLULAR MATRIX ORGANISATION, BOTH OF WHICH ARE CRITICAL FOR THE MAINTENANCE OF EPITHELIAL HOMEOSTASIS.

VINCULIN AND RNA BINDING PROTEINS IN REGULATING CELL ADHESION, MIGRATION AND EPIDERMAL HOMEOSTASIS

1. Understanding the role of vinculin in maintaining stem cell homeostasis in skin

Vinculin is a mechano-coupling, protein that is found both at cell-cell (adherens) junctions and cell-substratum (focal) adhesions. Vinculin helps to link the actin cytoskeleton to the junctions at the cell membrane. Although, vinculin itself does not interact directly with integrins, it is thought to play key roles in focal adhesion assembly and cell adhesion. In response to the force, vinculin not only accumulates at integrin- and cadherin-containing adhesions, but also it bears the force and transmits it to the cytoskeleton. It acts as a docking protein for several focal adhesion partners and alpha-actinin at cell-cell junctions thereby regulating several signaling pathways induced by mechanical forces. In order to study the

roles of vinculin in keratinocytes, we generated a skin specific conditional knck out (KO) mouse. Detailed analysis of the KO revealed that these animals display defects in the hair follicle cycle (wherein the KO animals had an accelerated hair cycle) while the epidermal development was completely normal. Label retaining experiments performed by pulsing with BrdU and chasing for several weeks revealed that the bulge stem cells fail to maintain their quiescence in the KO, which may explain the continuous cycling of the hair follicles. We have also shown that the vinculin KO cells form abnormal cell-cell junctions that may contribute to aberrant hair follicle cycle through loss of contact inhibition. Thus, the question we are trying to address is how does the loss of vinculin, a mechano-transducer result in loss of stem cell quiescence? And what role if any, the stem cell niche may play in this? This study will help understand the underlying mechanism that affects the normal hair follicle cycle and the signaling required to maintain the quiescence of hair follicle stem cells.

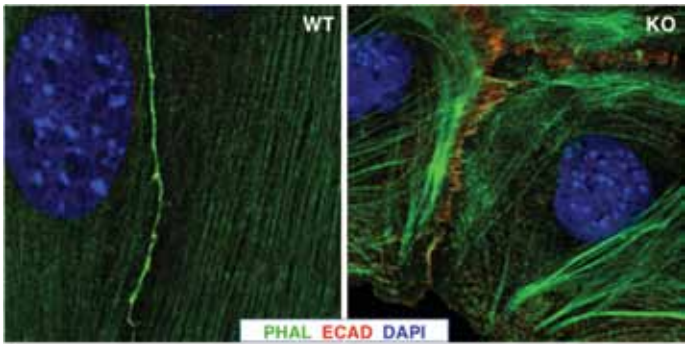


Figure 1: Abnormal cell-cell junctions in vinculin KO cells

2. Role of RNA binding proteins in regulating cell-substratum adhesion

The targeting of mRNAs and associated RNA binding proteins in non-neuronal cells has recently emerged as a possible mechanism to generate localized translation, at sites where the protein is required. One of the best examples of this is the "zipcode" sequence that is found in the β-actin mRNA that associates with the Zipcode Binding Protein ZBP-1 (an RNA binding protein). Recent studies have shown that ZPB1- targets the β actin mRNA on to focal adhesions where it mediates localized actin synthesis that modulates focal adhesion dynamics. We propose to investigate the role of local translation in generating and maintaining integrin mediated focal adhesions in keratinocytes, particularly at the early stage of cell spreading. We have identified several RNA binding proteins including PABPC1, HNPNPs, FUS, and DDX that are found in a complex with integrins in focal adhesions (preliminary data). Interestingly in a SILAC experiment that was reported in 2003 to identify new proteins that associate with focal adhesions in spreading cells, the Mann group identified a number of the same RNA binding proteins and showed that the loss of these proteins can perturb the process of cell spreading.

Our interest in these RNA binding proteins was reinforced as a result of our collaboration with the Dasaradhi Palakodeti (inStem), where we analyzed the phenotypes of PABPC1 knock down in *Planaria.* Poly A binding proteins (PAPBs) are RNA binding proteins which bind to mRNA poly A tails with their RRM domains. The function ofPABPC1 is highly conserved through evolution and it plays a major role in translation initiation with addional roles in post transcriptional modifications, RNA stabilization, nonsense mediated decay and miRNA mediated repression. The knockdown worms develop epithelial lesions, and upon closer examination we found that the epithelial layer is no longer associated with the underlying muscle layer, due to the loss of organization of the basement membrane (Bansal et al., in press). This phenotype is strikingly reminiscent of the β1 KO phenotype in

skin. We are therefore collaboratively exploring the function of PABPC1 in keratinocytes and epidermis in regulating ECM organization.

The aim of our project is to understand the role of PABPC in epithelial cells, particularly in the dynamics of cell-matrix adhesions and its implication in regeneration of the epithelial tissue, using both mouse keratinocytes and planariaas models.

3. Regulation of skin stem cells by small RNAs

Small RNAs have emerged as key players in gene regulation. This work aims to elucidate the role of one such class of small RNAs, tRNA-derived small RNA (tsRNAs), a rather poorly understood class of small RNA in the context of stem cell differentiation. The lab employs two different stem cell model systems to understand the role of these tsRNAs; the hair-follicular stem cells (HFSCs) that are adult stem cells and mouse embryonic stem cells (ESCs) which are pluripotent in nature (in collaboration with Ramanuj Dasgupta, GIS, Singapore, and Dasaradhi Palakodeti, inStem).

PUBLICATIONS

1. Kurbet A, Hegde S, Bhattacharya O, Marepally S, Vemula PK, Raghavan S (2016) Sterile Inflammation Enhances ECM Degradation in Integrin β1 KO Embryonic Skin. ***Cell Reports.*** (12):3334-47.

2. Bansal D, Kulkarni D, Nadahalli K, Lakshmanan V, Krishna S,Sasidharan V, Geo G, Dilipkumar S, Pasricha R, Gulyani A, Raghavan S, Palakodeti D (2017) Epidermal integrity critically regulated by cytoplasmic poly (A) binding protein (PABPC2) provides instructive cues for neoblast function during planarian regeneration. ***Development.*** 144(17):3066-3079.

HONORS & AWARDS

Gordon Research Conference on Epithelial Differentiation and Keratinization, Il-ciocco, Italy, May 2017 (session chair).

INVITED TALKS

1. Role of mechanical signaling in maintaining quiescence in mouse skin. Mechanical Forces in Cell Biology: Information at the Cell and Tissue Scale, Bangalore, October 2016.

2. Cell Adhesion in Skin. DBT-NER Workshop on Stem Cell Biology, Mumbai, January 2017.

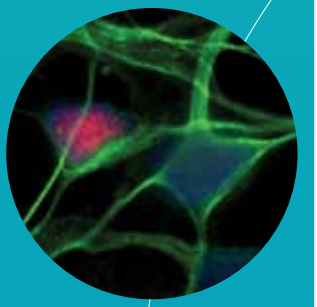
3. Role of mechanical signaling in maintaining quiescence in mouse skin. inStem Annual Talks, Bangalore, March 2017.

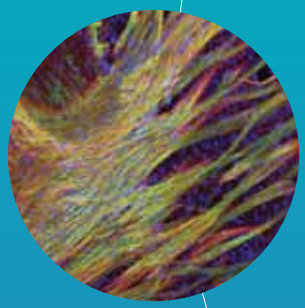
4. Breaking Barriers: Role of Integrins in Epithelial Homeostasis and Sterile Inflammation. Foster Lecture, Department of Physiology, Development and Neuroscience, University of Cambridge, UK, May 2017.

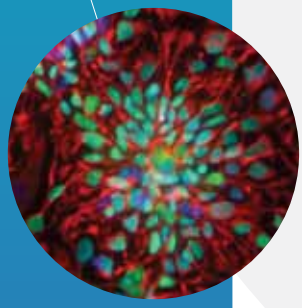
4

CBDR

CENTRE FOR BRAIN DEVELOPMENT AND REPAIR



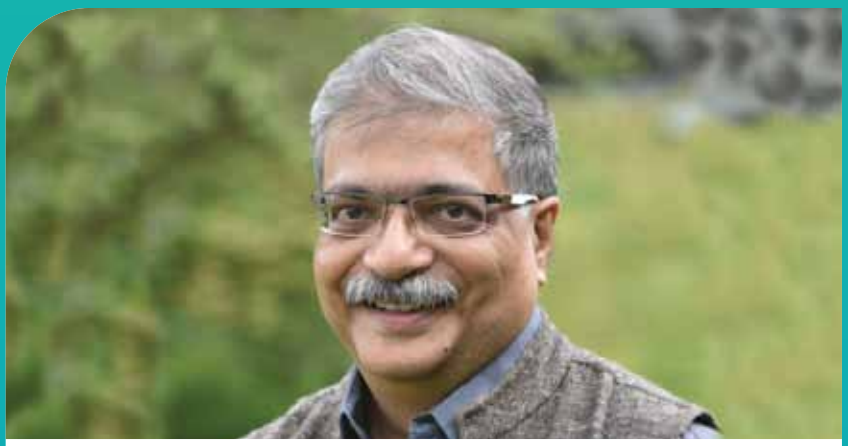




Neurodevelopmental and neurodegenerative brain disorders represent a major and growing public health threat. CBDR hosts two major programmes, the Centre for Neurodevelopmental Synaptopathies (CNS), an international collaborative centre between inStem, NCBS and the University of Edinburgh, and Accelerator Programme for Discovery in Brain Disorders using Stem cells (ADBS), a collaboration between inStem, NCBS and clinicians at the National Institute of Mental Health and Neurological Sciences (NIMHANS) at Bengaluru. The shared aim of these programmes is to accelerate the discovery and delivery of effective therapeutics for largely untreatable conditions. Although these are a disparate group of currently untreatable conditions that include acquired, developmental and ageing-related diseases, there are common themes and needs. Uniformly, the unmet need is for a human-based approach to investigating the causes, consequences and ultimately treatment of these diseases. To this end, CBDR has generated a range of expertise in several fields of neurobiology including synaptic function and plasticity, human stem cells and cognition behaviour. Theme activities are supported by grants from the Department of Biotechnology, the Wadhwani Foundation and Pratiksha Trust.

SUMANTRA CHATTARJI
Theme Coordinator

4.1



Sumantra Chattarji

shona@ncbs.res.in

CENTRE FOR NEURODEVELOPMENTAL SYNAPTOPATHIES (CNS)

NEURODEVELOPMENTAL AND NEURODEGENERATIVE DISORDERS POSE A MAJOR AND GROWING PUBLIC HEALTH THREAT. OUR RESEARCH SPANS MULTIPLE LEVELS OF NEURAL ORGANISATION AND COMBINES ANIMAL AND HUMAN-BASED MODEL SYSTEMS TO ACCELERATE THE DISCOVERY AND DELIVERY OF EFFECTIVE THERAPEUTICS FOR THESE LARGELY UNTREATABLE CONDITIONS.

Programme 1: Modelling human ASDs "in a dish"

Siddharthan Chandran, David Wyllie, Sumantra Chattarji

While genetic heterogeneity in Autism Spectrum Disorders and Intellectual Disability (ASD/ID) appears to be the rule, a high degree of convergence is also evident at the level of cellular/developmental processes and biochemical pathways, suggesting that relatively few targets for pharmaceutical interventions may provide functional benefit for a wide range of ASD/ID. Specifically, in light of recent studies of highly penetrant single gene syndromes that exhibit ASD/ID as part of their core features, we are testing the hypothesis that many of these genetic syndromes may be placed at different points of a common axis of synaptic pathophysiology. Thus, we are modelling ASD/IDs by generating novel human and rodent platforms (described later) to study forebrain neurons, synapses and circuits, alongside existing mouse models that will target convergent pathways of ASDs. We are initially focusing on Fragile X Syndrome

(FXS) as well as mutations in key glutamate receptors and their primary signalling pathways as prototypic causes of ASDs. To this end, a major new platform at CBDR uses human induced pluripotent stem cell (iPSC) based *in vitro* systems for both scientific discovery of cellular and synaptic mechanisms underlying ASD/ID and as a potential high throughput screening for pharmaceutical compounds. To this end, generation, maintenance and propagation of human iPSCs, neural conversion and cortical neurons are all in place in our centre (Figure 1A).

Using a combination of whole-cell patch clamp recordings and microscopy, we are investigating if human cortical neurons recapitulate known physiological and anatomical milestones and (Figure 1B-C), if so, whether these milestones are reached in human iPSC-derived neurons from affected individuals. Further, in the broader context of cellular autonomy and ASDs, we are studying the role of astrocytes in the development and function of forebrain neurons derived from FXS patient iPSC lines.

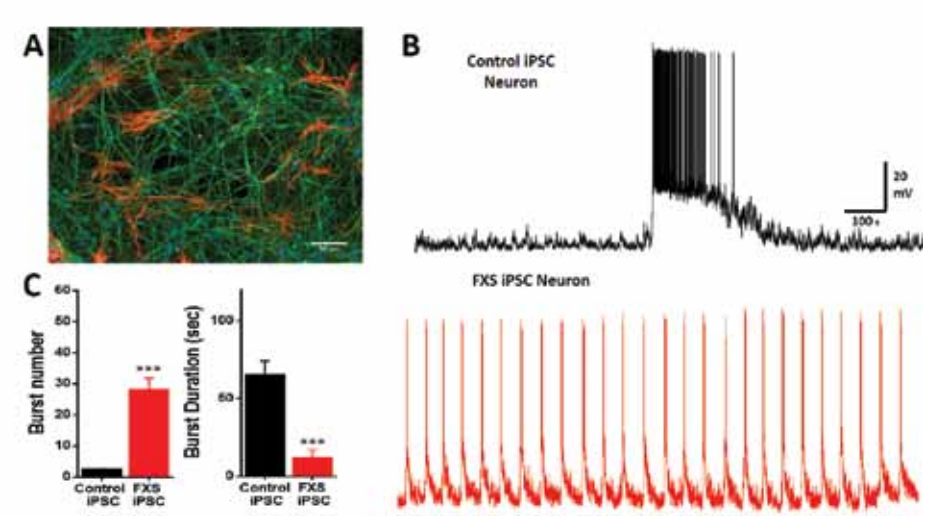


Figure 1: (A) Human iPSC-derived cortical neurons (green, Map2ab-positive) from a Fragile X Syndrome (FXS) patient co-cultured with rodent corticalastrocytes (orange, GFAP-positive) for 8 weeks *in vitro*. (B) *Top:* Burst of action potentials fired by control cortical neurons. *Bottom:* Burst of action potentials fired by FXS cortical neurons. (C) Quantification of action potential burst properties recorded from control and FXS cortical neurons

Programme 2: The autistic network – from pathways to rescue

Sumantra Chattarji and Peter Kind

Using novel transgenic rat models of ASDs, we are addressing whether genetically heterogeneous disorders share common synaptic neuropathology, as well as whether the common synaptic pathophysiology that arises from shared "developmental" mechanisms could be a therapeutic target throughout the lifespan of the animal. For instance, do rare, highly penetrant forms of ID with co-occurring ASD share a common time-course of cellular circuit-level defects? We have also completed a detailed characterisation of post- and pre-synaptic defects in the amygdala of both mouse and rat models of FXS. Identification of deficits in activity-dependent synaptic plasticity in the amygdala, in turn, has enabled us to examine their functional consequences at the systems/behavioural levels.

Programme 3: Autistic function – rat behaviour and imaging

Peter Kind,Sumantra Chattarji

We continue to generate new rat models of highly penetrant single-gene causes of ASD/ID to better model autistic and cognitive behaviours that can accurately reflect autistic features in humans. Rats are preferable in this regard to mice as they have a

wider repertoire of social behaviour. They also permit the use of functional magnetic resonance imaging (fMRI) in awake, behaving animals that then allows parallel studies in rodents and humans using the same modality. In a recently a published study from CBDR, using a new rat model of FXS, we report that *Fmr1*-KO rats exhibit elevated basal protein synthesis and an increase in mGluR-dependent long-term depression (LTD) in hippocampal area CA1 that is independent of new protein synthesis. These defects in plasticity are accompanied by an increase in dendritic spine density selectively in apical dendrites and subtle changes in dendritic spine morphology of CA1 pyramidal neurons. Behaviourally, *Fmr1*-KO rats show deficits in hippocampal-dependent, but not hippocampal-independent, forms of associative recognition memory indicating that the loss of FMRP causes defects in episodic-like memory. In contrast to previous reports from mice, *Fmr1*-KO rats show no deficits in spatial reference memory reversal learning. One-trial spatial learning in a delayed matching to place water maze task was also not affected by the loss of FMRP in rats. This is the first evidence for conservation across mammalian species of cellular and physiological phenotypes associated with the loss of FMRP in the hippocampus. Furthermore, while key cellular phenotypes are conserved, they manifest in distinct behavioural dysfunction. Finally, our data reveal novel information about the selective role of FMRP in hippocampus-dependent associative memory. In parallel, we have initiated a range of behavioural studies to test how models of ASD/ID show alterations in working memory, forgetting, social behaviours and repetitive behaviours.

Co-Principal Investigators

Siddharthan Chandran

Peter Kind

David Wyllie

PUBLICATIONS

1. Rahman MM, Kedia S, Fernandes G, Chattarji S (2017) Activation of the same mGluR5 receptors in the amygdala causes divergent effects on specific versus indiscriminate fear. ***Elife.*** 30;6. pii: e25665.

2. Thangaraj Selvaraj B, Livesey MR, Chandran S (2017) Modeling the C9ORF72 repeat expansion mutation using human induced pluripotent stem cells. ***Brain Pathology.*** 27, 4, 518-524.

3. Thomson SR, Seo SS., Barnes SA, Louros SR. Muscas M, Dando O, Kirby C, Hardingham GE, Wyllie DJA, Kind PC, Osterweil EK (2017) Cell type-specific translation profiling reveals a novel strategy for treating fragile X syndrome. ***Neuron.*** 2; 95(3):550-563.e5.

4. Yasmin F, Saxena K, McEwen BS, Chattarji S (2016) The delayed strengthening of synaptic connectivity in the amygdala depends on NMDA receptor activation during acute stress. ***Physiological Reports*** 4 (20), pii: e13002.

5. Rahman MM, Callaghan CK, Kerskens CM, Chattarji S, O'Mara SM (2016) Early hippocampal volume loss as a marker of eventual memory deficits caused by repeated stress. ***Scientific Reports.*** 4;6:29127.

6. Qiu J, Bilican B, Dando O, Magnani D, Livesey M, Kind PC, Simpson I, Wyllie DJA, Lowell S, Chandran S, Hardingham GE & 12 others (2016) Evidence for evolutionary divergence of activity-dependent gene expression in developing neurons. ***Elife.*** 1;5. pii: e20337.

7. Livesey M, Magnani D, Cleary EM, Vasistha N, James OT, Thangaraj Selvaraj B, Burr K, Story D, Shaw CE, Kind P, Hardingham G, Wyllie D, Chandran S (2016) Maturation and electrophysiological properties of human pluripotent stem cell-derived oligodendrocytes. ***Stem Cells.*** 34(4):1040-53.

8. Crocker-Buque A, Currie SP, Luz LL, Grant SG, Duffy KR, Kind PC, Daw MI (2016) Altered thalamocortical development in the SAP102 knockout model of intellectual disability. ***Hum Mol Genet.*** 25(18):4052-4061.

9. Bhattacharya A, Mamcarz M, Mullins C, Choudhury A, Boyle RG, Smith DG, Walker DW, Klann E (2016) Targeting Translation Control with p70 S6 Kinase 1 Inhibitors to Reverse Phenotypes in Fragile X Syndrome Mice. ***Neuropsychopharmacology*** 41, 1991–2000.

10. Bowling H, Bhattacharya A, Klann E, Chao M (2016) Deconstructing brain-derived neurotrophic factor actions in adult brain circuits to bridge an existing informational gap in neuro-cell biology. ***Neural Regen. Res.*** 11, 363.

11. Márkus NM, Hasel P, Qiu J, Bell KFS, Heron S, Kind PC, Dando O, Simpson TI and Hardingham GE (2016) Expression of mRNA Encoding Mcu and Other Mitochondrial Calcium Regulatory Genes Depends on Cell Type, Neuronal Subtype, and Ca2+ Signaling. ***PLoS One*** 11, e0148164.

12. Hector RD, Dando O, Landsberger N, Kilstrup-Nielsen C, Kind PC, Bailey MES, Cobb SR (2016) Characterisation of CDKL5 Transcript Isoforms in Human and Mouse. ***PLoS one*** 11, e0157758.

INVITED TALKS

1. The Amygdala in Health & Disease. Gordon Research Conference. Stonehill College, USA, 2017.

2. Stress and Cognition – From Basic Mechanisms to Psychopathology. Radboud Summer School on Nijmegen, The Netherlands, 2017.

3. The Simons Initiative for the Developing Brain. Patrick Wild Centre, University of Edinburgh, UK, 2017.

4. Stress: Past, Present and Future Directions. Princeton Neuroscience Institute, USA, 2017.

5. The Herrenhausen Symposium on Psychiatric Disorders (organized by Nature Medicine, Nature Neuroscience and the Volkswagen Foundation),. Hannover, Germany, 2016.

4.2

Ravi Muddashetty

ravism@instem.res.in

RNA MEDIATED REGULATION OF PROTEIN SYNTHESIS DURING NEURONAL DEVELOPMENT

WE HAVE IDENTIFIED THREE DISTINCT RNA MEDIATED MECHANISMS INVOLVING FMRP THAT PLAY IMPORTANT ROLE DURING NEURONAL DEVELOPMENT. THEY ARE FMRP-SNORNA INTERACTION AFFECTING RIBOSOME HETEROGENEITY, FMRP INTERACTION WITH NMD MACHINERY AFFECTING PLURIPOTENCY, AND FMRP INTERACTION WITH miRISC REGULATING SYNAPTIC PROTEIN SYNTHESIS.

The central focus of my lab so far has been to understand the role of FMRP during neuronal differentiation and development. Surprisingly, this quest has taken us from the synapse to the nucleus to the nucleolus and back. While we were studying the role FMRP as a modulator of synaptic protein synthesis in association with microRNA and RNA induced silencing complex (RISC), we discovered that FMRP also interacts with a specific subset of small nucleolar RNAs (snoRNAs). Through this interaction, FMRP alters the methylation of ribosomal RNA and generates heterogeneity in ribosomes. These heterogeneity marks are recognized by FMRP itself in the cytoplasm to regulate the translation of its target mRNAs. We also discovered that FMRP regulates the expression and function of nonsense mediated decay machinery (NMD) which plays a critical role in the cell proliferation of embryonic stem cells (ESC) and their

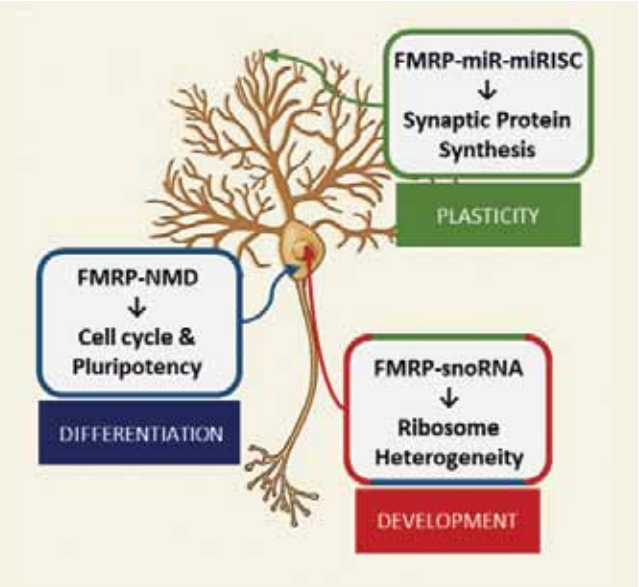


Figure 1: Three distinct RNA mediated mechanisms involving FMRP play critical role during neuronal development

commitment to differentiate into the neuronal lineage. While these are very distinct functions and may seem unrelated, we are now recognizing the threads that may connect these functions to describe multiple layers of translation regulation with FMRP as a subtle but critical guiding factor from differentiation to plasticity (Figure 1).

We have recently identified a novel interaction of FMRP with a class of small nucleolar RNAs (snoRNAs) which affects the methylation of ribosomal RNA (rRNA). We established that the 2'O methylation of rRNA guided by C/D box snoRNAs in human embryonic stem cells (hESCs) is incomplete at several sites which generate ribosome heterogeneity. FMRP interacts with a subset of C/D box snoRNAs targeting the sites which are only partially methylated. FMRP preferentially associates with ribosomes which are hypo or hyper methylated on specific sites on rRNA. Methylation status of these sites also alters the translation efficiency of the corresponding ribosomes. In the absence of FMRP, rRNA methylation is affected particularly on sites which are partially methylated and thus alters ribosome heterogeneity. Hence, FMRP not only alters the methylation but also recognizes specific methylation patterns on ribosomes, thus determining the mRNAs translated by these ribosomes. Interestingly, FMRP interacts with the same set of snoRNAs in human ESCs, ESC derived neuronal precursor cells (NPC) and HeLa cells indicating a conserved function of FMRP across different cells. In contrast, FMRP interacts with different sets of microRNAs (and potentially mRNAs) in ESCs, NPCs, and neurons. Hence, FMRP interaction with snoRNAs is likely to determine the ribosomes which will regulate the translation of FMRP target mRNAs which may be cell and developmental stage specific. We have also established that FMRP interacts with nonsense mediated decay (NMD) machinery in hESCs and this interaction changes when they differentiate into NPCs. This plays an important role in determining the rate of cell cycle and maintenance of pluripotency and thus influences neuronal differentiation. We are currently investigating the correlation between ribosome heterogeneity generated by FMRP and its effect on NMD mediated regulation of FMRP target mRNAs during neuronal development.

Finally, taking our study back to synapse where we started from, we have identified that the microRNAs and miRNA induced silencing complex (miRISC) have a central role in regulating synaptic protein synthesis. We show that reversibility of miRISC mediated inhibition is a common mechanism to regulate translation downstream

of both mGluR and NMDAR stimulation. Using rat cortical synaptoneurosomes and cultured neurons 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 the phosphorylation status of FMRP acts as a molecular switch downstream of both the receptors. While dephosphorylation of FMRP promotes translation on mGluR stimulation, it is phosphorylation of FMRP inthe case of NMDAR stimulation. Using ribosome profiling from synaptoneurosomes, we have identified a distinct set of mRNAs which are translationally activated by NMDAR and mGluR at a genome-wide scale. Analysis of the results from ribosome profile indicates an interesting overlap of transcripts regulated by NMDAR and mGluR with the genes implicated in autism spectrum disorders (ASD). We are currently studying the impact of NMDAR and mGluR stimulation on energy dynamics and its correlation to protein synthesis at the synapse. To further understand the functional significance of different domains of FMRP, we have generated several FMRP mutants based on SNPs identified in patients having fragile X like phenotypes. We are now studying these mutants for their transport to different compartments of neurons, interaction with translation machinery, and response to stimulation. These results clearly highlight the broader interest of studying the molecular mechanisms of synaptic translation regulation for the field of Fragile X syndrome (FXS) and ASD research.

INVITED TALKS

1. Stem cell models to understand fragile X syndrome. Indian Academy of Neuroscience, National Center for Brain research, Manesar, September 6, 2016.

2. When and Where? the problem of protein synthesis in FXS. School of Regenerative Medicine- Manipal University, February, 28, 2017.

4.3

Mahendra Rao
mahendrar@instem.res.in

Raghu Padinjat
praghu@ncbs.res.in

ACCELERATOR PROGRAMME FOR DISCOVERY IN BRAIN DISORDERS USING STEM CELLS

THIS PROGRAMME USES MODERN STEM CELL TECHNOLOGY TO CREATE CELLULAR MODELS OF THE BRAIN DERIVED FROM HUMAN SUBJECTS WITH A FAMILY HISTORY OF MENTAL ILLNESS. THE OVERALL GOAL IS TO UNCOVER THE GENETIC, CELLULAR AND MOLECULAR BASIS OF MENTAL DISORDERS.

Psychiatric disorders are a major source of disability in young adults with about 2- 3% of the population at risk for developing these disorders both in India and across the world. These disorders are recognized as one of the major non-communicable diseases (NCD) and a significant contributor to morbidity as articulated by the World Health Organization's New Delhi call for action on combating NCDs in India. Given the large number of individuals affected by mental illness, the development of novel therapies will likely have important positive social and economic benefits. There is a pressing need to understand the mechanistic basis of these disorders so that such information can be translated into novel diagnostic and therapeutic approaches.

Mental illnesses are recognized as having an inherited basis. However, despite their high heritability and the identification of a large number of 'common' and rare variants, few genetic correlates of mental illness have been identified. Many of the genes (and pathways)

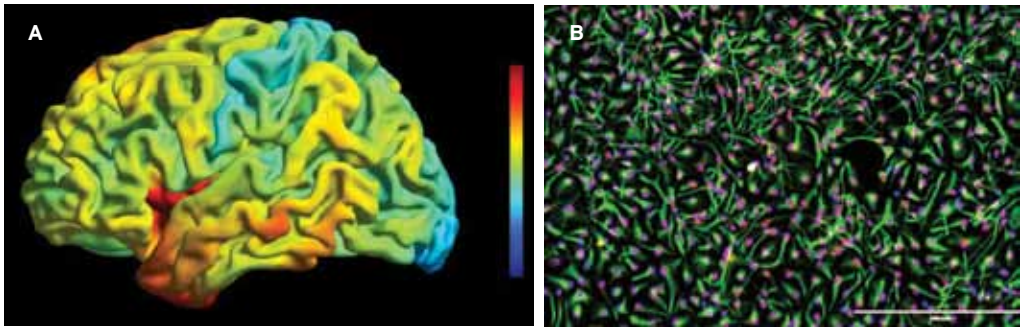


Figure 1 (A): Rendered and colour coded cortical thickness map derived from the structural MRI image (T1-weighted) of a human brain (blue to red colour gradient indicates increasing range of values in mm). Structural MRI studies help to measure various global and regional metrics (e.g. volume, cortical thickness, surface area and gyrification index) of the human brain non-invasively. This in turn can shed light on potential neuroanatomical correlates of the brain in health and disease.

Figure 1 (B): Cultured neural stem cells (NSC) generated from a human subject in the ADBS program. NSC are stained to show the expression of markers of neural stem cells. Nestin (green) is a cytoplasmic protein. SOX2 (Red) is a nuclear marker that co-localizes with DAPI that marks DNA.NSC can be differentiated in a dish into neurons and glial cells whose function can be studied experimentally. Such "disease in a dish models" can help uncover the cellular basis of altered brain function in mental illness.

identified suggest aberrant neural development and connectivity in early life as being critical to their pathogenesis. The epigenetic changes that occur due to environmental exposure during windows of sensitivity in the developing brain, give rise to different trajectories of brain development and lead to variations in temperament, response to stress and substance abuse.

Given the gene/environment interactions that over time are likely to lead to psychiatric disorders, well-defined, prospective clinical cohorts offer a unique opportunity to understand the pathogenesis of mental disorders. In collaboration with the Department of Psychiatry, National Institute of Mental Health and Neurosciences (NIMHANS), we have identified a prospective cohort of families with a high density of mental illness. These families will be followed over a period of twenty years to map the development of disease through detailed clinical investigations (Figure 1A) at regular intervals. In addition, we will establish stem cell lines of affected individuals and unaffected controls from these families (Figure 1B). This material will be used to generate cellular models of neural cells in which the mechanistic aspects of cellular neurobiology and physiology that may lead to disease can be studied. This work will be done in collaboration with colleagues from the National Centre for Biological Sciences (NCBS).

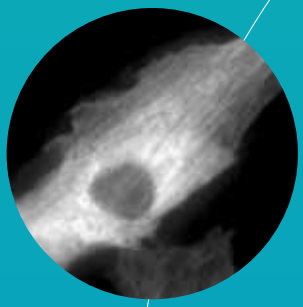
Collectively, we aim to understand the relationship between cellular phenotypes and disease progression. This will be accomplished through collaborative research applying modern genetic analysis and cell-based assays on patient derived cell lines in conjunction with information from detailed clinical analysis.

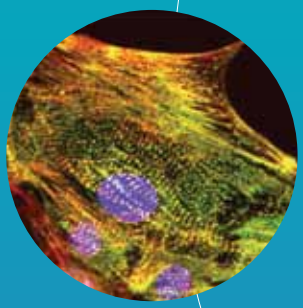
THE ACCELERATOR PROGRAM FOR DISCOVERY IN BRAIN DISORDERS USING STEM CELLS (ADBS) IS A NEW SCIENTIFIC VENTURE TO UNDERSTAND MENTAL ILLNESS BY HARNESSING THE POWER OF MODERN HUMAN GENETICS AND STEM CELL TECHNOLOGY.

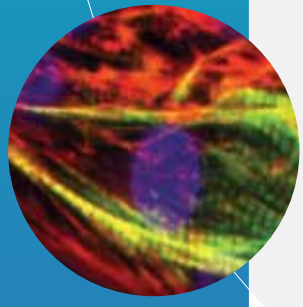
5

CCBD

CENTRE FOR CARDIOVASCULAR BIOLOGY AND DISEASE







The Centre for Cardiovascular Biology and Disease theme at inStem, focuses on the signalling and biomechanical properties of the heart, with an initial emphasis on genetic hypertrophic and dilated cardiomyopathies, and autosomal dominant myocardial diseases caused by missense mutations primarily in one of the several genes encoding the fundamental contractile apparatus of the heart. These diseases are common, debilitating and often lead to sudden death. This theme brings together a team of scientists using complementary approaches to a fundamental clinical issue in India and worldwide. Interactions and collaborations across our team members are strong, bringing together biochemistry, biophysics, biology, genetics, structural biology, computational biology and clinical sciences to define how cardiomyopathy mutations affect the power output of the human heart. Our ultimate goal is to understand the underlying molecular mechanisms of hypertrophic and dilated cardiomyopathies in order to develop new therapeutic approaches for these diseases.

JAMES SPUDICH
Theme Coordinator

SIVARAJ SIVARAMAKRISHNAN
Theme Coordinator

5.1

Minhaj Sirajuddin

minhaj@instem.res.in

NANOENGINEERING FOR DISCOVERY AND DISEASE MECHANISM RELATED TO CYTOSKELETON

EUKARYOTIC BIOLOGICAL MOTIONS ACROSS SCALES AND ORDERS OF MAGNITUDE INVOLVE CYTOSKELETON ELEMENTS AND MUTATIONS IN THEM ARE FREQUENTLY ASSOCIATED WITH HUMAN PATHOLOGY, e.g., CARDIOMYOPATHIES, NEUROLOGICAL SYNDROMES AND CILIOPATHIES. WORK FROM OUR LAB, UTILIZES THE POWER OF NANOENGINEERING AND *IN VITRO* RECONSTITUTION TO UNCOVER NEW FINDINGS IN CYTOSKELETON BIOLOGY, BRIDGING THE KNOWLEDGE GAP BETWEEN CLINICAL FINDINGS AND MOLECULAR MECHANISM.

As a part of the cardiomyopathy team, my research will focus on bridging the knowledge gap between clinical findings and molecular mechanism underlying cardiomyopathy disease causing mutations. Together, our theme covers human genetics, animal models, cell biology, biochemistry and structure at the molecular level of cardiomyopathy diseases. Currently our group is focusing on reconstituting a minimal contractile unit and crystal structures of sarcomere proteins implicated in cardiomyopathy. Both these project will benefit from the collaborative research environment of CCBD theme and Bangalore Bio-cluster campus.

A major hurdle in achieving reconstitution of minimal contractile unit is generating arrays of myosin motors that approximate the order found in a half-sarcomere (i.e., one half of a bipolar thick filament). Engineering a defined number of myosin motors and understanding their cooperativity during force generation represent a fundamental challenge in muscle biology and motor biophysics. One of ourmain goals is to engineer thick myosin filaments with precise control over the number of myosin heads and their topology. In addition to addressing fundamental questions in the muscle field, the reconstituted system will allow us to study cardiomyopathy mutations and their effects in force generation during muscle contraction. Here we have utilized the self-assembling DNA origami system to achieve a near native structure myosin thick filaments (dimeric myosin heads displayed in a helical array). For designing a synthetic hemi-thick filament assembly we are using computational methods to design a modular assembly to achieve a 400 nm and 800 nm wide DNA structure with precise topology of attachment sites for the dimeric myosin heads (Figure 1). We have successfully folded the designed DNA origami structure and validated it using negative stain electron microscopy (Figure 1).

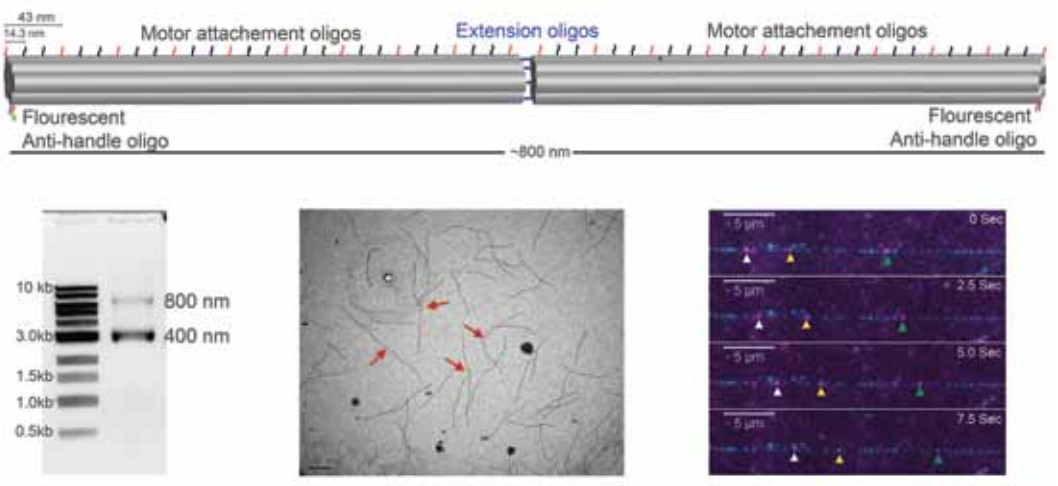


Figure 1: Illustration of DNA origami module design. The spacing of handle sites are similar to native myosin thick filaments (top). Agarose gel of folded 400 nm and 800 nm DNA origami structures (bottom, left). Electron micrograph of folded DNA origami structures shows 400 nm and 800 nm (red arrows) DNA origami structures, (bottom, middle). Motility images of DNA structure: motor complex moving on filament tracks (bottom, right).

Simultaneously, we have developed methods to attach SNAP tagged motors to a linear DNA assembly and performed motility assays (Figure 1). On the myosin motor front, we have established methods to attach native myosin heads to the DNA. Further steps will involve measuring the processivity and velocity of myosin assemblies. In addition to titrating the number of myosin heads, the DNA origami system allows the possibility to vary motor spacing and add stalling elements (e.g., rigor myosin).

Several proteins connect the thin and thick filaments and the Z-disc units to each other and mutations in these proteins have also been implicated in cardiomyopathies. For example, titin links thick filaments and Z-disc, myosin binding protein links thick and thin filaments, α-actinin from the Z-disc forms an anchoring point for thin actin filaments. New evidence indicates that the Z-disc not only provides a boundary, but also contributes to stabilization of sarcomeres, mechanosensation and signal transduction. There are over a dozen proteins present in the Z-disc structures and mutations in every

single component lead to a variety of diseases, including HCM and DCM. Among them, nexilin is the least characterized of the proteins implicated primary cardiomyopathies. Clinical studies have identified 5 mutations that cause HCM or DCM that are clustered at the amino- and carboxy-terminal of nexilin respectively.

Structure-function studies have been initiated, to characterize both structural and biochemical changes resulting from the mutations and understand tissue specific functions of Nexilin. Our preliminary efforts in this direction include, purification of full-length, truncated and mutant nexilin proteins and actin binding assays. We closely collaborate with Dhandapany Perundurai's lab to identify new mutations in nexilin and other sarcomere proteins, including a a novel mutation in nexilin present in the Indian population, identified by his laboratory. Future work will involve introducing this mutation in to our structure-function pipeline and mice model.

In addition, to the sarcomere related work, our lab is working towards understanding the microtubule cytoskeleton, in particular, tubulin posttranslational modification (PTM) and organization of specialized microtubules in cilia and flagella. Our recent work on this field has uncovered fundamental principles that regulate dynein motility during intracellular cargo transport (McKenney et al., 2016). We are currently collaborating with Carsten Janke from Curie Institue, Orsay/Paris, France to tackle tubulin PTMs. Our combined effort using *in vitro* reconstitution, cell biology and mouse models will be an important step towards understanding how microtubule growth, dynamics and stability is governed by tubulin PTMs.

PUBLICATIONS

McKenney RJ, Huynh W, Vale RD, Sirajuddin M (2016) Tyrosination of α-tubulin controls the initiation of processive dynein-dynactin motility. ***EMBO J*** (2016) 35(11):1175-85.

INVITED TALKS

1. Regulation of microtubule motors by tubulin modifications. Max-Planck-Institute, Dortmund, Germany, June 2016.

2. Regulation of microtubule motors by tubulin modifications. Curie Institute, Orsay, France, June 2016.

HONORS & AWARDS

1. EMBO Young Investigator Program (2017 – 2021)
2. CEFIPRA (2017 – 2020)

5.2

Dhandapany Perundurai

dhan@instem.res.in

GENES, MECHANISMS AND THERAPIES FOR CARDIOMYOPATHIES

THE LONG-TERM GOAL OF MY RESEARCH GROUP IS TO EXPLORE NEW GENES, MECHANISMS AND RELEVANT DRUGS THAT HAVE SIGNIFICANT CLINICAL AND CURATIVE IMPACT ON CARDIOMYOPATHIES (FIGURE 1)

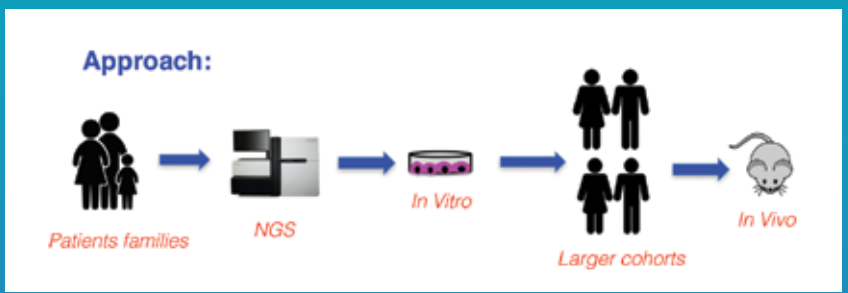


Figure 1: The schematic shows the organization of work elements in our laboratory. Towards the broader goal of advancing precision medicine our approaches can be divided into the following steps: identification of genes associated with HCM; interrogate underlying mechanisms and assess possibilities for therapeutic intervention.

A. Gene discovery for South Asian specific cardiomyopathy patientsB.

(i) We have recently organized 190 unrelated Indian cardiomyopathy index patients

participants) for our genetic studies. These patients are selected because they are negative for known (reported) genes and will enhance the probability of identifying the new gene(s). We performed exome sequencing in 60 selected participants (30 index patients and their 30 unaffected family members respectively as internal controls). Various bioinformatics tools are used to identify genes associated with cardiomyopathy.

(ii) New genes are identified including *ADIPOR1.*

(iii) The new gene *(ADIPOR1)* obtained is sequenced in a large cohort comprising 600 Indian cardiomyopathy patients and 500 controls to establish the frequency and specificity of the novel mutation respectively. We found this gene is responsible for 1% of cases.

B. Molecular mechanisms associated with AdipoR1 (AR1) variant (F145I)

To know the functional consequences and to determine, whether expressing the AdipoR1 mutated proteins ($AR1^{F145I}$) in cardiomyocytes promote hypertrophy (modeling the HCM phenotype observed in patients), various parameters including cell surface area, fetal gene expression, and protein synthesis rate were analyzed. Transient expression of the mutant protein in rat cardiomyocytes (RCMs) showed a significant increase in cell sizes, fetal genes expression including atrial natriuretic factor (ANF), brain natriuretic peptide (BNP) and β-myosin heavy chain (βMHC) compared to wildtype AdipoR1 ($AR1^{WT}$). Protein synthesis rates, assayed with [^{3}H]-leucine incorporation, were also elevated in mutant overexpressing myocytes compared to control wild type. Collectively, these data suggest constitutive expression of $AR1^{F145I}$ promotes cardiac hypertrophy.

To dissect the signaling pathways modulated by $AR1^{F145I}$, we assessed its effect in the lysates obtained from the cardiomyocytes overexpressing $AR1^{WT}$ and $AR1^{F145I}$ using immunoblotting for various downstream targets. Surprisingly, the well-known downstream targets of AR1 including AMPK/LKB1/ACC were relatively normal in the cardiomyocytes expressing mutant compared to WT. However, we found evidence that $AR1^{V146M}$ selectively target the p38/mTOR pathway members. The mutant expressing RCM showed increased phosphorylation of p38, mTOR, 70 kDa ribosomal protein S6 kinase ($p70^{S6K}$) and eukaryotic elongation factor-2 kinase (eEF2K). Also, treatment of known inhibitors of p38 or mTOR rescued the cardiomyocyte hypertrophy and restored the normal signaling in mutant -expressing cells, respectively.

C. Humanized transgenic mice models of cardiomyopathies

(i) Characterization of a mouse model of Adiponectin Receptor R1 *(ADIPOR1):* We generated a transgenic mouse model with a novel ADIPOR1 mutation in collaboration with Djamel Lebeche (Mount Sinai School of Medicine, New York). The mice are viable and develop cardiomyopathy around 12 weeks. The histological analyses in these mice hearts revealed a massive cardiomyopathy with the hallmarks of hypertrophy including increased cells sizes and myocardial fibrosis. In addition, the cardiomyocytes obtained from these mice showed increased p-38/mTOR activity similar to heart biopsy obtained from the patient suggesting a gain of function effect.

(ii) Establishing Cardiac Myosin Binding Protein (MYBPC3) mouse model: Our previous work on the molecular genetics of cardiomyopathies led to several discoveries

including an ancient common variant (25bp deletion) associated with cardiomyopathies in MYBPC3 in South Asians. This variant, in its homozygous nature, causes severe childhood cardiomyopathies. Now, we have generated a humanized cardiac specific transgenic mouse models for this variant using standard Cre-loxP recombination methods. We obtained five viable founder lines and are in the process of characterizing the physiological, functional and molecular aspects of this mouse model.

(iii) Drug discoveries and molecular mechanisms in the mice: Both the above -outlined mice (*MYBPC3* and *ADIPOR1*) models provide an opportunity to validate the drugs and its mechanisms for cardiomyopathies. The treatment of ADIPOR1 mouse model with Rapamycin rescues the cardiomyopathy phenotypes.

D. Unraveling *RAF1* signaling in cardiomyopathy

RAS-MAPK pathway plays a crucial role in the structural and functional development of myocardium. Gene mutations in RAS-MAPK pathway members leading to its aberrant signaling are a major cause of childhood onset cardiomyopathies in syndromic (RASopathies) or non-syndromic patients. We identified *RAF1* (a member of RAS-MAPK) as a predominant cause of childhood dilated cardiomyopathy. We are exploring the molecular mechanisms of these mutants including the pathway specific interplay between Raf1 and sarcomeric proteins using cellular and *Drosophila* models. The RAF1 mutant expression using UH3 specific drivers cause severe flightless defects and with tin-c driver cause cardiac hypertrophy. We are in the process of doing a protein array to find phospho-specific sites on the sarcomere.

PUBLICATIONS

1. Pandi-Perumal SR, BaHammam AS, Ojike NI, Akinseye OA, Kendzerska T, Buttoo K, Dhandapany PS, Brown GM, Cardinali DP (2016) Melatonin and Human Cardiovascular Disease. ***J Cardiovasc Pharmacol Ther.*** Jul 21 (in press).

2. Akinseye OA, Ojike NI, Akinseye LI, Dhandapany PS, Pandi-Perumal SR (2016) Association of Sleep Duration with Stroke in Diabetic Patients: Analysis of the National Health Interview Survey. ***J Stroke Cerebrovasc Dis.*** 25(3): 650-5.

INVITED TALKS

Novel genes associated with cardiomyopathies in South Asians. IIT-Chennai, 12th May 2017.

HONORS & AWARDS

Torrent Young Investigator Award at the 13th Annual Conference of International Society for Heart Research, Chennai.

5.3

John Mercer

jam@instem.res.in

UNDERSTANDING PRIMARY INHERITED CARDIOMYOPATHIES AT MULTIPLE LEVELS OF ORGANISATION

MUTATIONS IN ANY ONE OF THE GENES ENCODING THE PROTEINS THAT PRODUCE MUSCLE CONTRACTION CAUSE INHERITED PRIMARY CARDIOMYOPATHIES. OUR GROUP'S GOAL IS TO DETERMINE THE COMMONALITIES AND DIFFERENCES IN THE MECHANISMS BY WHICH THESE MUTATIONS CAUSE DISEASE.

The heart muscle is made up of specialised contracting cells called cardiomyocytes. Each cardiomyocyte consists of bundles of myofibrils that have a characteristic striped or striated appearance, formed by repeating sarcomeres. The sarcomere is the fundamental structural and functional unit of muscle, comprised of interdigitating thick and thin filaments. Myosin, the molecular motor that powers muscle contraction, comprises the thick filament and the thin filament is made up of multiple proteins: actin, tropomyosin and troponins. Force generation during contraction is achieved by the sliding movement of the thick and thin filaments relative to each other, triggered by calcium.

Primary cardiomyopathies are disorders of the heart muscle, in the absence of any other disease. They affect 1 in 500 people. The best-described causes are the hundreds of different missense mutations in any one of the genes encoding the cardiac sarcomeric proteins. In response to the primary dysfunction, the morphology of the heart is remodelled, most often along one of two different patterns, with the walls becoming thicker (hypertrophic cardiomyopathy or HCM) or thinner (dilated cardiomyopathy or DCM). The heart muscle is made up of specialized contracting cells called cardiomyocytes. Each cardiomyocyte consists of bundles of myofibrils that have a characteristic striped or striated appearance, formed by repeating sarcomeres. The sarcomere is the fundamental structural and functional unit of muscle, comprised of interdigitating thick (the molecular motor myosin) and thin filaments (actin, tropomyosin and troponins). Force generation during contraction is achieved by calcium triggering the sliding movement of the thick and thin filaments relative to each other.

Mutations in genes encoding the proteins that produce muscle contraction cause inherited primary cardiomyopathies, affecting 1 in 500 people. In response to the primary dysfunction caused by these mutations, the morphology of the heart is remodelled, most often along one of two different patterns, with the walls becoming thicker (hypertrophic cardiomyopathy or HCM) or thinner (dilated cardiomyopathy or DCM).

Having studied a set of tropomyosin mutants, we have leveraged our experience to study a set of troponin T (TnT) mutants. TnT is a component of the calcium-sensing machinery in muscle, and disease-causing mutations are concentrated in the region of TnT (TNT1, shown in Figure 1) that interacts with tropomyosin. We are collaborating with R Sowdhamini (NCBS), whose group has great expertise in studying these types of protein structures. Our studies, which have been submitted for publication, show that mutations at one end of troponin T affect contractility by the same mechanism: changing binding affinity for tropomyosin. This knowledge provides a route to identifying small therapeutic molecules that modulate this affinity.

At the organismal level, we have characterized our humanised mouse model, expressing human cardiac myosin in place of mouse cardiac myosin, to study the mutants at the level of the whole organism. Despite the very different biochemical properties of the two myosins, the mice expressing the human myosin are surprisingly normal in their

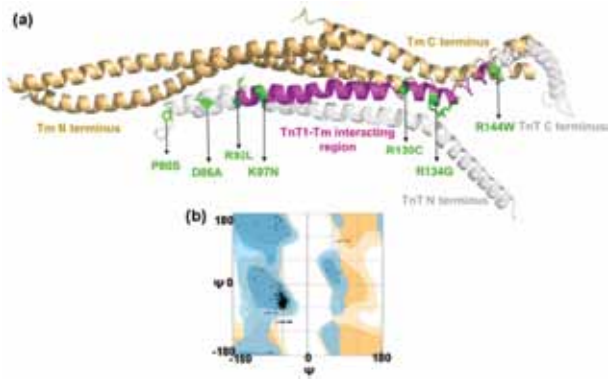


Figure 1: Illustration of interaction of the TNT1 domain of troponin T with tropomyosin (Tm).

a. 131 residues of N- and C-terminal Tm (sand color) overlap structure with the TNT1 architecture. The TNT1-Tm interaction zone is shown in magenta. All the mutants are distributed and labeled according to their positions in green.

b. Ramachandran map.

cardiac phenotypes, making them an attractive platform for studying the human mutations in the human protein. This characterisation was performed in collaboration with N Ravi Sundaresan (IISc, Bengaluru) and is close to submission for publication.

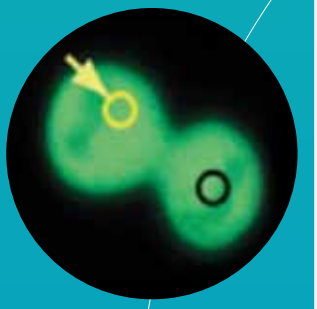
PUBLICATIONS

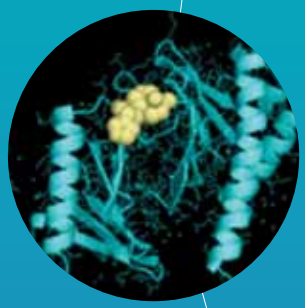
1. Gangadharan B, Sunitha MS, Mukherjee S, Chowdhury RR, Haque F, Sekar N, Sowdhamini R, Spudich JA, Mercer JA (2017) Molecular mechanisms and structural features of cardiomyopathy-causing troponin T mutants in the tropomyosin overlap region. ***Proc Natl Acad Sci USA*** (in press).

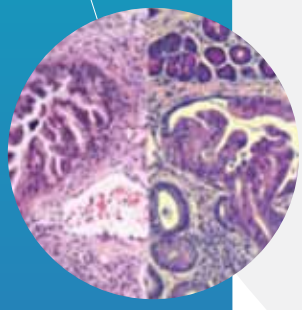
6

CCBT

CENTRE FOR CHEMICAL BIOLOGY AND THERAPEUTICS







The Centre for Chemical Biology and Therapeutics (CCBT) is an integrated, multidisciplinary programme whose central aim is to develop innovative approaches to create chemical tools that modulate novel classes of targets, in order to explore the fundamental biological mechanisms underlying human diseases like cancer. Our long-term vision is not only to provide novel insights into disease mechanisms, but also to translate this new knowledge into the discovery of novel approaches for therapy. We expect our work to provide a framework for chemical biology and translational research across the campus. To facilitate this, the CCBT has been constituted as an inter-institutional collaborative Centre between NCBS and inStem.

ASHOK VENKITARAMAN
Theme Coordinator

6.1

Ashok Venkitaraman

ashokv@ncbs.res.in

THE CENTRE FOR CHEMICAL BIOLOGY AND THERAPEUTICS: TOWARDS NEXT-GENERATION MEDICINES

THE CENTRE FOR CHEMICAL BIOLOGY AND THERAPEUTICS (CCBT) PIONEERS INNOVATIVE APPROACHES TO CREATE CHEMICAL TOOLS THAT MODULATE NOVEL CLASSES OF TARGETS, IN ORDER TO EXPLORE THE FUNDAMENTAL BIOLOGICAL MECHANISMS UNDERLYING HUMAN DISEASES, WITH THE LONG-TERM VISION TO SEED THE DISCOVERY OF NEW THERAPEUTICS.

The CCBT's first scientific focus is to explore new approaches for the modulation of intracellular signaling pathways disrupted in disease, by targeting the molecular recognition of key classes of post-transcriptional protein modifications. Our first objective is to create a unique palette of selective chemical tools that modulate the recognition of site-specific protein phosphorylation by specific domains.

1. We have explored three structural paradigms for the molecular recognition of phosphopeptide substrates during intracellular signal transduction. Protein domains recognizing pSer, pThr (tandem (t)BRCT domains from BRCA1 or ECT2) or pTyr (the SH2

domain from GRB2) have been expressed, purified and structurally characterized in their apo- or ligand-bound forms using computational modelling and X-ray crystallography. This work has identified key features underlying molecular recognition by the tBRCT domain family, which provides insight into the discrimination of pSer versus pThr, and exposes characteristics underlying selective substrate recognition by different family members (*Bharatham* et al., manuscripts in preparation).

2. A focused chemical library comprising ~130K elements has been designed, sourced and organized in an appropriate LIMS system. High-throughput primary screening assays (using fluorescence polarization) with Z scores ≥0.6 against different domain targets were developed, and over 1 million in vitro screening reactions have been completed. Over 400 active compounds that selectively inhibit molecular recognition by either the BRCT or SH2 domains were identified, and taken forward into hit validation through orthogonal assay development for different domain targets.

3. Our first programme targets the BRCA1 tBRCT domain, which is representative of tBRCT family members that selectively recognize phosphopeptide substrates containing hydrophobic side chains proximal and distal to pSer, through interactions with an extended binding groove at the interface between the two BRCT motifs. Similar domains occur in evolution from bacteria to man, in proteins serving biological functions in DNA replication and repair. We have:

(i) Identified 4 structural clusters of compounds that target the BRCA1 tBRCT, and developed binding mode hypotheses for each cluster using computational chemistry.

(ii) Verified the binding mode hypothesis for the most promising cluster through a cascade of iterative chemical synthesis and biophysical assays, synthesizing and testing >100 novel compounds exploring 3 positions (Figure 1) to identify key elements contributing to the structure-activity relationship (SAR).

(iii) Developed a series of novel chemical leads that selectively engage the BRCA1 tBRCT domain with *in vitro* Kd ~75nM, and favourable physico-chemical properties for further optimization. These compounds exemplify the first potent non-peptidic inhibitors of substrate recognition by the tBRCT domains *(Potluri, Bharatham, Goyal, Padigaru, Sadasivam et al., patent in preparation).*

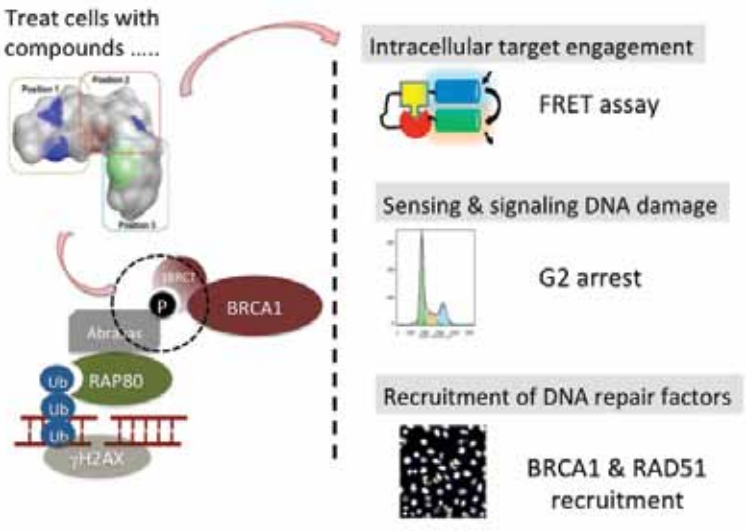


Figure 1: Binding mode hypothesis for the most promising cluster and inhibition of the G2 cell cycle checkpoint for DNA damage, and of the assembly of DNA repair proteins at sites of DNA damage

(iv) Determined the structure of the apo- and phosphopeptide-liganded forms of the BRCA1 tBRCT domain at <3Å, in order to determine the binding mode of lead inhibitors through soaking or co-crystallization.

(v) Established a series of new assays that monitor tBRCT target engagement in the cellular milieu, combining the cellular thermal shift (CeTSA) and FRET approaches, which provide a blue-print for cellular testing of compounds that target protein-protein interactions *(Sadasivam et al., manuscript in preparation).*

(vi) Demonstrated that example compounds from our novel chemical lead series induce biological responses consistent with their mechanism of action (Figure 1) via the BRCA1 tBRCT, including inhibition of the G2 cell cycle checkpoint for DNA damage, and of the assembly of DNA repair proteins at sites of DNA damage. These compounds exemplify selective chemical leads that target the functions of the BRCA1 tBRCT in cells *(Bharatham, Goyal, Padigaru, Potluri, Sadasivam et al., manuscript in preparation).*

4. Our second programme targets the tBRCT domain of ECT2, a component of protein complexes that control guanine-nucleotide hydrolyzing enzymes during mitotic cell division and protein synthesis. There is relatively little structural information concerning this poorly studied tBRCT domain, but available information suggests that the second (carboxyl-terminal) BRCT motif in the pair is twisted by ~90° compared to that in the BRCA1 tBRCT. We have identified in preliminary studies 5 active compounds that competitively inhibit phosphopeptide substrate binding by ECT2 tBRCT, and bind directly to the domain with Kd ~3-10μM.

5. Our third programme targets the SH2 domain of GRB2, an accessory protein involved in signal transduction via receptor tyrosine kinases (RTKs). This target exemplifies a large family of SH2 domains that selectively recognize pTyr in the context of particular motifs surrounding the phosphorylated residue, which are critical for cellular regulation. We have identified in preliminary studies 2 active compounds that competitively inhibit phosphopeptide substrate binding by GRB2 SH2, and bind directly to the domain with Kd ~50μM.

The overall outcome of these programmes will be to deliver, in a stepwise fashion, a palette of novel chemical probes that selectively modulate the molecular recognition of pSer, pThr or pTyr-containing substrates in order to systematically explore how this mechanism contributes to intracellular signaling. These programmes will also establish a powerful inter-disciplinary capability for chemical biology and therapeutics development in the inStem/NCBS campus.

INVITED TALKS

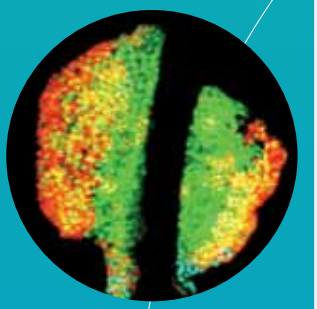
1. Extending the reach of target discovery for cancer and other diseases. A*STAR, Singapore, October 2016.

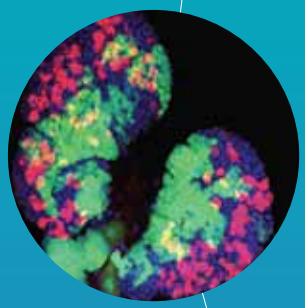
2. Early intervention in cancer through the tumour suppressive mechanisms that control genome stability. Stanford University, CA, USA, August 2016.

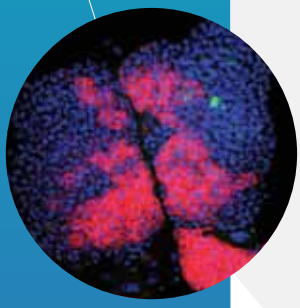
7

RCF

REGULATION OF CELL FATE







The dynamic modulation of cellular repertoires in tissues requires a diverse set of distinct cell fate decisions that are shaped by the local microenvironment as well as systemic cues. Emerging evidence suggests that in the context of homeostasis, the efficacy of short and long-range signals is influenced by organismal physiology and in turn by intracellular metabolic states. Perturbations of homeostasis consequent to cell turnover, injury, infections or the deletion of damaged/defective cells are reset by the activation of tissue-resident, specialized stem/progenitor cells through changes in their intracellular signaling and metabolic programs. Growing evidence supports a key role for metabolic state as a determinant in diverse cell fate choices. Metabolic checkpoints are key regulators of cellular responses via integration of information among intracellular, tissue-level and body-wide physiological events. The theme "Regulation of cell fate" accommodates a range of activities aimed at understanding responses to physiological and pathological challenges to tissue homeostasis; an important focus will be on an inter-connected investigation of metabolic control of cell fate, using diverse models.

APURVA SARIN
Theme Coordinator

7.1

Apurva Sarin

sarina@instem.res.in

METABOLIC SIGNALING UNDERLYING CELL FATE DECISIONS IN T-CELLS

METABOLIC REPROGRAMMING IN MATURE T-CELLS IN THE MAMMALIAN IMMUNE SYSTEM, IS INTERTWINED WITH DECISIONS OF DIFFERENTIATION AND HOMEOSTASIS. WE ARE INTERESTED IN MOLECULAR UNDERPINNINGS OF METABOLIC PLASTICITY, NECESSITATED BY THE CHANGING NICHES THAT T-CELLS FUNCTION IN.

Tuning function in response to nutrient availability is an evolutionarily ancient cellular response. In T-cells, a cell type in the mammalian immune system, negotiating nutritional stress is critical for lineage stability and requires metabolic reprogramming. Aligned with the goals of the RCF theme, our research is focused on identifying metabolic underpinnings of cell fate decisions (and function) in the T-cell lineage. Specifically, we seek to understand how known determinants of cell fate integrate with cellular metabolic signaling, with a focus on the regulation of cell survival. Towards this aim, ongoing lines of research include, the identification of sentinel metabolites and metabolic sensors and the interplay with known regulators of T-cell functional fates, to query if a particular metabolic state is key for the maintenance of the differentiated fate.

Several years ago, we had shown that signaling from the developmental regulator, Notch, protected cells from a wide variety of stressors that trigger cell death. Protective Notch1

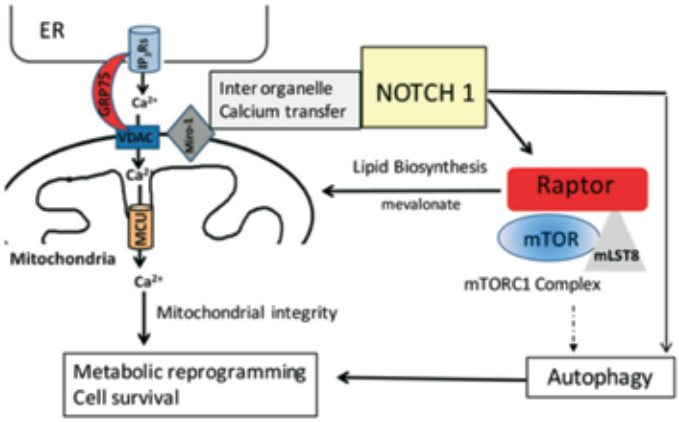


Figure 1: The schematic, (not drawn to scale), depicts, recent research directions in the laboratory. These have emerged from our efforts to understand Notch integration of metabolic programming in Tregs. Our current focus is centred on Mitochondria and the cross-talk with Notch activity

signaling was coupled to its localization and subsequently, we uncovered a requirement for this pathway in the maturation and functioning in the T-regulatory cell (Treg) subset in the immune system (Marcel & Sarin 2016). Our more recent work, has identified mitochondria as a key target of Notch activity. Mitochondria are an important checkpoint as they serve to integrate cell survival and apoptotic or cell death cues.

While Notch activity is critical for Treg adaptation to limiting nutritional cues, our experiments have shown that the converse is also true. Thus, metabolic changes triggered by nutrient stress, are critical for localization of Notch. These experiments originated from observations in a cell-line based screen, which identified Sirtuin (Sirt)-1, a NAD^+-dependent deacetylase as a regulator of Notch activity. Sirtuins are enzymes that react to changes in energy levels and are implicated in metabolic homeostasis. Sirt1 activity controlled Notch localization, i.e. retention of the processed intermediate in the cytoplasm. Not unexpectedly, Sirt1 and NIC are detected as immune-complexes in Tregs, which is consistent with the evidence of functional interactions identified in our experiments. Further, Notch-dependent Treg suppressor activity, assessed *in vivo,* was also sensitive to Sirt1 modulation. Taken together, the experiments suggest that the Sirt1-Notch interaction may constitute a checkpoint controlling Notch1 signaling and immune function.

Calcium is a well-known second messenger implicated in a wide array of signaling processes. It is not surprising therefore, that apoptotic signaling frequently correlates with changes in the levels and distribution of intracellular calcium pools. Calcium overload in mitochondria triggered by apoptotic stimuli leads to the activation of signaling cascades culminating in cell death. However, regulated uptake of calcium in mitochondria can increase bioenergetic output via the enhance activity of calcium dependent reactions in the organelle. We asked if the distribution and regulation of cellular calcium is tuned by Notch activity. Specifically, our experiments test the hypothesis that regulation of calcium homeostasis (through utilization or active removal of excess calcium) in mitochondria could be a means of altering mitochondrial activity and cellular adaptation to stressors, associated with Notch activity. The endoplasmic reticulum (ER) is the major cellular store of cellular calcium and its connections with other organelles and molecules controlling the movement of calcium are relatively well understood. We have begun characterizing the cellular calcium landscape and functions of molecules at the ER and mitochondria in response to Notch activity. We find that Notch activity reduced free calcium in the ER store, also evident from blunted calcium release into the cytoplasm. Intriguingly,

we find that Notch activity confers a dependence on the mitochondrial calcium uptake machinery for the regulation of ER calcium levels and inhibition of apoptotic damage. Our experiments include a well-characterized anti-apoptotic protein from the Bcl-2 family and are yielding intriguing insights into differing mechanisms activated for signaling survival in cells.

Other research directions arising from our work, pertain to cross-talk of Notch with the cellular nutrient sensing machinery as well as sentinel metabolites, are currently under investigation in the laboratory.

PUBLICATIONS

1. Marcel N, Perumalsamy LR, Shukla SK, Sarin A (2017) The lysine deacetylase Sirtuin 1 modulates the localization and function of the Notch1 receptor in regulatory T cells. ***Sci Signal.*** 10(473) aah4679.

2. Sarin A, Marcel N. (2017) The NOTCH1-autophagy interaction: Regulating self-eating for survival. ***Autophagy.*** 13(2):446-447.

3. Marcel N and Sarin A (2016) Notch1 regulated autophagy controls survival and suppressor activity of activated murine T-regulatory cells. ***ELife*** 5:e14023.

INVITED TALKS

Notch signaling controls pathways regulating cellular homeostasis.
Biotechnology interventions in human health: infection, immunity and inflammation.
12th Indo-Australia Biotechnology Conference, Institute of Life Science, Bhubaneshwar, August 7-9, 2016.

7.2

Arjun Guha

arjung@instem.res.in

REGULATION OF LUNG REPAIR

WE ARE BROADLY INTERESTED IN THE PROCESSES THAT PROTECT AND REPAIR THE LUNG. WE FOCUS ON THE MECHANISMS BY WHICH THE EPITHELIAL LINING OF THE RESPIRATORY TRACT COPES WITH DAMAGE CAUSED BY EXPOSURE TO CHEMICAL AND BIOLOGICAL TOXICANTS.

Epithelial tissues line the surfaces of organs throughout the body and serve to both nourish and protect. The maintenance and post-injury repair of these tissues is of vital importance. Some epithelia, such as the skin and the lining of the alimentary canal, have high rates of cell proliferation and turnover during homeostasis. In contrast, others such as the lining of the respiratory tract in the lung have significantly lower rates but can dramatically upregulate proliferation in response to injury. We focus on the regulation of the epithelial stem/progenitor cells in the lung.

The laboratory is currently investigating a rare population of multipotent epithelial progenitor cells (Uroplakin 3a (Upk3a)$^{+}$ Club cells (U-CCs) that are distributed throughout in the lower respiratory tract but enriched at specific locations. These are Neuroepithelial Bodies at airway branch points (NEBs) and Bronchioalveolar Duct Junctions (BADJs). We have shown that U-CCs can contribute to the maintenance/repair of the airways and to the post-injury repair of the alveoli (see graphic below). Our goal is to identify the signals in the NEB/BADJ microenvironments that regulate the fate of U-CCs during injury-repair.

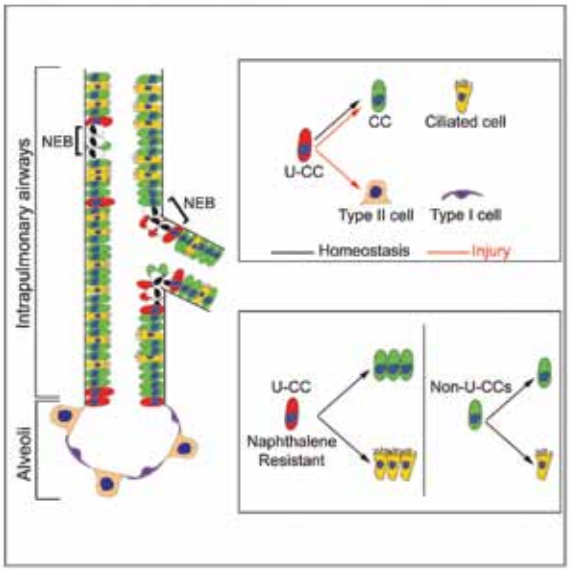


Figure 1: The cartoon is a summary of findings from lineage analysis of Uroplakin 3a+ Club cells (U-CCs) in the adult mouse lung. U-CCs contribute toward airway maintenance and post-injury repair and generate both Club and ciliated cells. These progenitors can also contribute toward alveolar repair post Bleomycin injury.

Together with studies in the mouse model, we are also interested in the regulation of the progenitors of the adult respiratory system (tracheal system) of *Drosophila.* The transformation from a free-living larva to an adult fruit fly is dependent on several adult epithelial progenitor populations that remain mitotically inactive until the onset of metamorphosis. Our goal is to delineate the mechanisms for cell cycle progression in progenitors of the adult tracheal system. In the long term we will investigate whether the regulatory mechanisms are also utilized in the vertebrate lung.

PUBLICATIONS

1. Guha A, Deshpande A, Jain A, Sebastiani P, Cardoso WV (2017) Uroplakin 3a+ cells are a distinctive population of airway progenitors that contribute to airway maintenance and post-injury repair. ***Cell Reports.*** 19 (2), 246 – 254.

INVITED TALKS

Epithelial stem/progenitor cells and niches in the lower lung. Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, USA, September 1, 2017.

7.3

Sunil Laxman

sunil@instem.res.in

METABOLIC SENSING AND REGULATION OF CELL FATE

THE METABOLIC STATE OF A CELL CAN DIRECTLY CONTROL DIFFERENT CELL FATE DECISIONS. CERTAIN METABOLITES REFLECT METABOLIC STATE, AND ARE SENSED BY "METABOLIC SENSORS". OUR GROUP IDENTIFIES HOW SPECIFIC METABOLITES (PARTICULARLY AMINO ACIDS), AND THEIR METABOLIC SENSORS REGULATE CELL FATE, AND DISCOVERS THE PROCESS BY WHICH SUCH INFORMATION IS TRANSFERRED WITHIN CELLS.

A cell can undergo different fates ranging from division, differentiation and autophagy to cell death. It is now apparent that the metabolic state of a cell itself controls these cell fate decisions. The metabolic state depends upon central metabolites, and their sensing by specific "metabolic sensors", which work by controlling metabolic master-switches. These metabolic sensors are finely tuned to sense important central metabolites, which control metabolic responses. Our research identifies sentinel metabolites and their metabolic sensors, and investigates the process of information transfer that they mediate within or between cells, in order to regulate cell fate. We are especially interested in how cells sense and respond to amino acids, as a fundamental question in cell biology. We have embarked on distinct projects that address these questions directly, and use model organisms (particularly *S. cerevisiae)* to discover these conserved processes, by using a combination of genetic, biochemical, proteomic and metabolomic approaches.

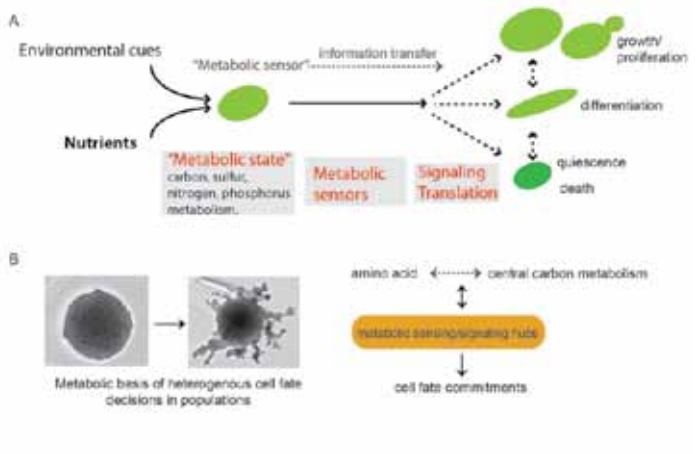


Figure 1: Metabolic sensing and regulation of cell fate

A) A schematic illustrating the process of metabolic sensing and information transfer resulting in diverse cell fates

B) Isogenic populations of cells differentiating and becoming to specialised communities

C) Integrating signals from amino acid and central carbon metabolism

Several research projects investigating the coupling of specific amino acid dependent processes, and central carbon metabolic processes, have matured, and have revealed novel, unexpected mechanisms by which cells control growth, differentiation, or heterogenous behaviour in populations. We have recently uncovered the biochemical basis of what makes methionine/s-adenosyl methionine (SAM) a special, "anabolic" metabolite. We can show that methionine alters anabolism by promoting reductive biosynthesis, and determine the mechanisms through which methionine/SAM carries out this function even in amino acid starved cells. This study (under review) has opened up multiple other directions in the lab, including the specific transcriptional regulation of amino acid homeostasis regulating genes, translational control dependent on metabolic state, and signaling events that sense and communicate metabolic state. In collaboration with Dr Sandeep Krishna, we have come up with theoretical physical models to explain how internal availability of specific metabolites can allow cells to oscillate between quiescent-like and proliferative states. In addition, we have also developed a substantial interest in amino acid based regulation of gluconeogenesis and the pentose phosphate pathway, and the function of these processes in regulating cell fate. Along the way, we have developed novel, highly sensitive, targeted and quantitative metabolite analysis methods, and ways to investigate nitrogen and carbon metabolic flux. Finally, we are exploring the design and development of *in vivo* sensors for several key metabolites. All this is now possible, due to a team of excellent postdoctoral fellows and PhD students, who drive individual projects as well as work on team efforts.

A note on collaborative efforts: within the theme, the collaboration with Apurva Sarin's group on amino acid sensing and regulation of T-cell function is maturing. In addition, we are collaborating with Tina Mukherjee's group on specific, focused projects stemming from our observations made in *S. cerevisiae.* The collaboration with Teymuras Kurzchalia (MPI-CBG, Dresden), on understanding the metabolic basis of desiccation tolerace in cells (Erkut et al, 2016), is formally continuing, with the hosting of a joint postdoctoral fellow.

PUBLICATIONS

1. Deshpande AA, Bhatia M, Laxman S, Bachhawat AK (2017) Thiol trapping and metabolic redistribution of sulfur metabolites enable cells to overcome cysteine overload. ***Microbial Cell,*** 4 (4), 112 – 126.

2. Laxman S (2017) Conceptualizing Eukaryotic Metabolic Sensing and Signaling. ***Journal of the Indian Institute of Science.*** 97: 59. (Invited Review).

PREPRINT:
3. Kumar S, Vaswata Roy Choudhury R, Laxman S (2017) Colonizer: Anandroid OS based automated microbial colony counter. ***PeerJ*** Preprints 5:e2792v1.

INVITED TALKS

1. Making commitments: how key metabolites determine cell proliferation decisions. CDFD, Hyderabad, India, March 2, 2017.

2. Building specialized cellular communities.Centre for Human Genetics, Bangalore, India, December 2, 2016.

3. Metabolic switching in regulating cell survival and growth. BSBE, IIT Bombay, Mumbai, India, June 15, 2016.

4. Metabolic switching in regulating cell survival and growth. DBS, Tata Institute of Fundamental Research, Mumbai, India, June 10, 2016.

5. A metabolic basis for building complex, specialized cellular communities in a "simple" eukaryote. SDB conference, IISER Pune. June 26, 2017.

6. Biochemical Evolution. Symposium on Evolutionary Biology, IIT-Bombay, Mumbai, India. April 1, 2017.

7. Metabolism and multicellularity. The Interface of Biology and Theoretical Computer Science, NCBS, Bangalore, India, Dec 19, 2016.

8. Physics of Life, 4th NCBS-Simons Monsoon School, Bangalore, India, June 22, 2016.

7.4

Tina Mukherjee

tina@instem.res.in

SYSTEMIC AND METABOLIC CONTROL OF HEMATOPOIESIS

OUR INTEREST LIES IN THE AREA OF INVESTIGATING MECHAINSMS COORDINATING METABOLISM AND DEVELOPMENT WITH SPECIFIC EMPHASIS ON UNDERSTANDING IMMUNE DEVELOPMENT.

USING *DROSOPHILA* BLOOD ORGAN AS THE PRIMARY MODEL SYSTEM WE ARE INVESTIGATING NEURONAL AND METABOLIC REGULATION OF BLOOD DEVELOPMENT.

Neuronal control of hematopoiesis

We aim to identify long-range signals from the brain regulating hematopoietic cell fate decisions. The nature of the signaling enitity, its sensing mechanism (whether through receptors or transporters), downstream signaling events and physiological relevance are key questions that we address under this objective.

In this area our work from over 2 years here at inStem has identified systemic metabolites derived from neurons that are integral to hematopoietic cell fate decisions. Specifically, upon parasitic wasp infections, *Drosophila* larvae respond by generating specialized cells termed lamellocytes that clear the deposited wasp eggs. We describe the adaptive use of the olfactory system in priming cellular immunity such that fly larvae with prior exposure to pathogenic

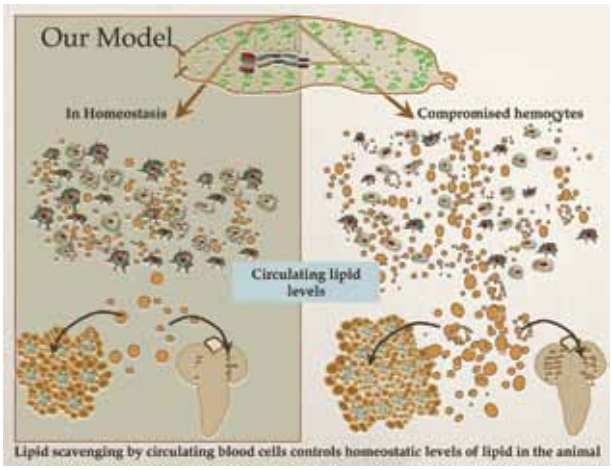


Figure 1: Lipid scavenging by circulating blood cells controls homeostatic levels of lipid in the animal

odours respond superiorly when challenged by the pathogen. Developmentally, odour dependent olfactory-receptor neuron (ORN) stimulation leads to systemic release of neuronal γ-Aminobutyric acid (GABA). This GABA is coopted by the immune system as an inflammatory metabolite. GABA catabolism in immune precursors stabilizes Hypoxia Inducible Factor (HIF)- alpha protein whose availability during infections dictates the strength of lamellocyte differentiation. Interestingly, predisposition to wasp odours leads to elevation of physiological GABA levels and consequently immune precursor HIF-alpha protein. These findings delineate a novel non-behavioural paradigm of odours sensing on priming cellular immune competency and innate immunity. These findings clearly imply that neuronal inputs are essential regulators of immune homeostasis in development and infections. We will explore this avenue in greater depth in the upcoming year.

Metabolic processes regulating hematopoiesis

Multiple lines of evidences across vertebrates and invertebrates have linked metabolic disorders such as diabetes and obesity with altered immune functions. While these are conflicting views of the involvement of immune cells in progression of these diseases, we are using a genetic screening platform to tease apart the connection between immune homeostasis and metabolism. To this end we have identified macrophages as global regulators of lipid homeostasis in addition to their canonical roles as immune effectors. Unlike the known functions of immune cells in obesity and metabolic disorders where active inflammatory signals or insulin resistance drives the pathology, our data indicates that these phenotypes are not due to perturbations in insulin signaling or activation of inflammation. These phenotypes are more suggestive of a homeostatic role played by macrophages in nutrient regulation. We expect the outcomes from our screening platform to help elucidate mechanistically on how immune cells respond to dietary changes.

INVITED TALKS

1. Maintaining Myeloid Cell Fate and Function Through Stress Sensing Pathways. Conflict and cooperation in cellular populations meeting, inStem, Bangalore, 17th October 2016.

2. Sensory Perception in Myeloid Cell Development and Function. NISER, Bhubaneshwar, 16th February 2017.

3. Sensory control of Myeloid Development and Function. Department of Biochemistry, AIIMS, New Delhi, 10th April 2017.

7.5

Jyotsna Dhawan

jdhawan@instem.res.in

QUIESCENCE AND ADULT STEM CELL POTENCY

OUR GROUP IS INTERESTED IN THE MECHANISMS BY WHICH THE DORMANT OR QUIESCENT STATE OF ADULT MAMMALIAN STEM CELLS PROMOTES THE ACQUISITION OF REGENERATIVE FUNCTION.

Most cells in adult tissue have ceased cell division, but can exist in distinct arrested states. Differentiated cells permanently withdraw from the cell cycle, but stem cells idle in a dormant state known as quiescence or G_0. These temporarily arrested progenitors maintain adult tissues undergoing normal turnover, as well as regenerate damaged tissue following injury. De-regulation of quiescence underlies pathologies at opposite ends of a spectrum- cancer may represent a failure to enter quiescence, while degenerative disease may represent a failure to exit quiescence. Therefore, understanding the acquisition and maintenance of quiescence has broad implications for human disease.

We use genome-wide strategies coupled with functional analysis to investigate the links between two key features of quiescence-repression of differentiation and the potential to return to active division. Using myogenic cell lines, muscle stem cells and mesenchymal stem cells we have described active controls at multiple levels of gene regulation specific to quiescence. Our studies indicate that quiescent cells preserve two antagonistic programs

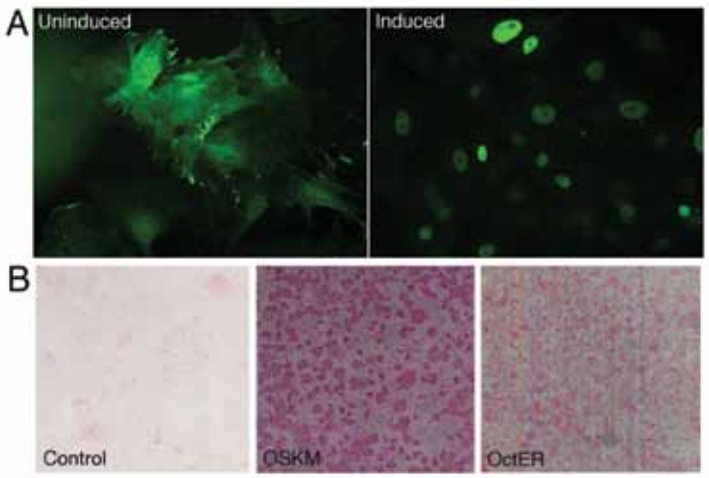


Figure 1: Reprogramming switch.

A. Uninduced cells express high levels of Oct4 in the cytoplasm (left panel) while addition of 100nM 4-hydroxy-tamoxifen induces nuclear localisation of OctER in mouse embryo fibroblasts (right panel).

B. OctER can replace Oct-3/4 to reprogram MEFs to iPSC. Alkaline phosphatase staining of colonies: *Control* represents untransduced MEFs (left panel), *OSKM* represents iPSC created using the original Yamanaka factors (middle panel) and *OctER* represents iPSC generated by replacing Oct-3/4 with the inducible OctER (right panel).

(division vs. differentiation) in an inactive but poised state that is rapidly altered by cell cycle reentry. Over the past year, we have continued our investigations into the molecular control of adult stem cell quiescence. Some highlights of these studies are:

Disease genes that affect quiescence

We are studying the links between redox stress and muscle disease in a model for a genetic disease, SEPN1-related myopathy (SEPN1-RM) in collaboration with Ana Ferreiro at CNRS. SEPN1 encodes the SelN protein and mutations lead to muscle atrophy. Using perturbations and transcriptome analysis in our culture model, we have found that SelN is required for effective reversible quiescence. SEPN1 knockout muscle stem cells show increased differentiation. Using rescue experiments with different regions of SEPN1 and drugs, our ongoing work suggests that newly identified pathways perturbed by loss of SEPN1 may represent therapeutic avenues for SEPN1-RM, potentially targetable with antioxidant or epigenetic drugs.

The relationship between cell fate and quiescence during reprogramming

Reprogramming of MEFs to an induced pluripotent state offers an excellent system to study stage-specific cell cycle control. We have developed an inducible reprogramming switch using a tamoxifen-dependent Oct-3/4 (Figure 1). Our results suggest that Oct-3/4 and Klf-4 synergize in the dismantling of the somatic cell cycle and the cell fate change involving the Mesenchymal to Epithelial transition (MET) during early reprogramming.

PUBLICATIONS

1. Sowpati DT, Srivastava S, Dhawan J, Mishra RK (2017) C-State: An interactive web app for simultaneous multi-gene visualization and comparative epigenetic pattern search. ***BMC Bioinformatics*** (in press).

2. Srivastava S, Gala H, Mishra RK, Dhawan J (2016) Distinguishing states of arrest: Genome-wide descriptions of cellular quiescence using ChIP-seq and RNA-seq analysis. ***Methods in Molecular Biology.*** Daniel Lacorazza (Ed) (in press) (Invited article)

3. Vyas N, Dhawan J (2016) Exosomes: mobile platforms for targeted and synergistic signaling across cell boundaries. ***Cell. Mol. Life Sci.*** 74(9):1567-1576.

4. Sellathurai J, Nielsen J, Dhawan J, MFB Nielsen, HD Schrøder (2016) Low oxygen tension enhances expression of myogenic genes when human myoblasts are activated from G0 arrest. ***PLOS One*** 21;11(7):e0158860.

INVITED TALKS

1. Still life-the quiescent genome and RNA pol II pausing. FASEB Summer Research Conference on Muscle Stem and Satellite Cells, Keystone Colorado, USA, July 2016.

2. Quiescence in stem cells: how can dormant cells contribute to tissue repair? Indian Association for the Cultivation of Science Kolkata Colloquium, Sep 2016.

3. RNA polymerase and the transcriptional program in G0: Does the quiescent myoblast capture essential features of the quiescent satellite cell? Institut Pasteur, Paris, Dec 2016.

4. Choosing cell fate: Balancing options, timing inactivity. Keynote lecture at Simons Center for Living Machines symposium on The interface of biology and theoretical computer science, NCBS, Dec 2016.

5. Quiet Time In Stem Cells: The Balancing Act of Reversible Arrest. Colloquium at IISER, Tirupati, 2017.

6. The quiescent genome: Balancing options, timing inactivity. Asian Chromatin Meeting, Hyderabad, Mar 2017.

7. Still life: the quiescent stem cell. YIM-Goa, Mar 2017 (India Biosciences).

8. Sleeping Stem Cells and Tissue Regeneration. National Conference on Biotechnology and Environment, Jamia Millia Islamia, New Delhi, April 2017.

9. The quiescent genome: Balancing options, anticipating change. Center for DNA Fingerprinting and Diagnostics, Hyderabad, April 2017.

HONORS & AWARDS

1. Member, Research Advisory Council, Center for DNA Fingerprinting and DNA Technologies.

2. Member, Scientific Advisory Committee, World without GNE Myopathy.

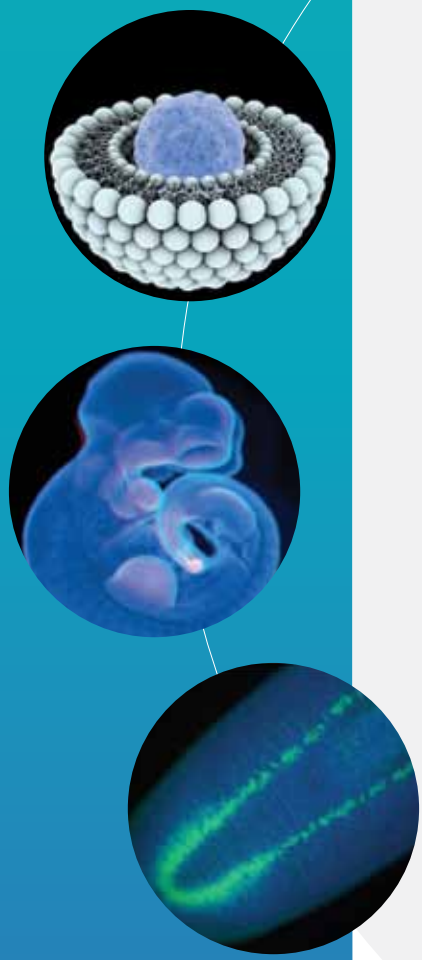
3. Member, Board of Governors TransDisciplinary University, Bengaluru.

8

TAS

TECHNOLOGIES FOR THE ADVANCEMENT OF SCIENCE

The TAS theme develops technologies and catalyzes new approaches to solve difficult problems in fundamental and applied biology. As part of these efforts, we span scales - understanding and tweaking processes from the molecular level all the way to level of whole organisms. A defining feature of the TAS theme is a culture of collaboration. We work together extensively within the group, the institute and the wider scientific community. For instance, we are adapting and developing the planarian model to address a frontier area of biology – regeneration - in new ways. Along this journey, we have made fundamental discoveries in natural light sensing and post-transcriptional regulation that have been widely recognized. Our light sensing research sheds new light on eye evolution, while helping map functional eye-brain regeneration. We have developed new materials for biomaterial delivery that have helped develop the planarian model, while impacting biomedical research (inflammation alleviation). Regeneration is intimately linked to development. Our work to understand cues and signals that control the body plan and cell fate also led to the practical discovery of making specific types of muscles cells from stem cells in a dish. We have solved new structures (including studying *in-vivo* crystals), developed new fluorescent tools and made breakthroughs in understanding novel RNA-mediated regulation. Overall, our achievements showcase how we integrate and transcend disciplines through our unique, bold and collaborative vision.



S RAMASWAMY
Theme Coordinator

8.1

S Ramaswamy
ramas@instem.res.in

Jeff Abramson
jabramson@mednet.ucla.edu

MOLECULAR FORM AND FUNCTION PROGRAM

IT HAS BEEN ALMOST FIVE YEARS SINCE JEFF JOINED INSTEM AS A COLLABORATIVE SCIENCE CHAIR. THESE FIVE YEARS HAVE BEEN VERY EXCITING AND HAVE HAVE BEGUN TO YIELD MANY ACCOMPLISHMENTS. SOME OF THESE ACHIEVEMENTS (A LARGE COLLABORATIVE PROJECT) INCLUDE THE ATOMIC RESOLUTION STRUCTURE OF THE FIRST NINE-CARBON SUGAR TRANSPORTER AND ITS FUNCTIONAL CHARACTERIZATION AT THE MOLECULAR LEVEL. IN ADDITION, STRUCTURE-FUNCTION STUDIES OF TWELVE DIFFERENT PROTEINS IN THE SIALIC ACID METABOLISM PATHWAY HAVE BEEN ACCOMPLISHED. MANY PROJECTS ARE NOW FULLY DEVELOPED WITH STUDENTS AND POST-DOCS IN THEIR LAST LAPS WITH HIGHLY SIGNIFICANT AND INTERESTING RESULTS ON THE HORIZON. ON A MORE PERSONNEL FRONT, THE FIRST GRADUATE STUDENT FROM THE GROUP COMPLETED PhD (2016) AND SEVERAL MORE TO COME.

In the last report, we had mentioned that our paper on the nutritious value of cockroach milk protein was accepted for publication (Banerjee et al., 2016). After publication, a new set of experiments have demonstrated that we can produce this protein synthetically in yeast – demonstrating that our work is now ready for translation. The other project that has now

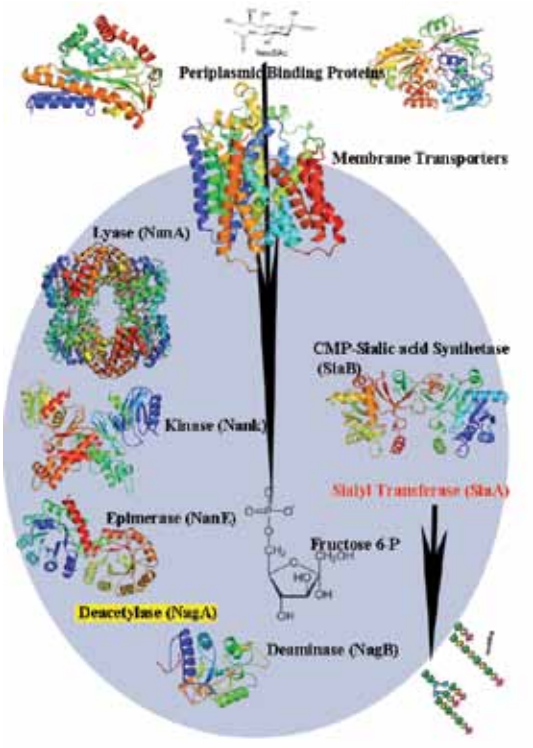


Figure 1: The various fates of Sialic acid in the gram negative bacteria. These sugars areused both as a carbon source as well as incorporated in the LOS as the outermost sugar. All structures shown here are determined by us.

We are still struggling to phase the structure of the Nucleotide Sugar transporter proteins, but we can now reproduce crystals that consistently diffract to about 3.0Å resolution. We hope that in the next report we will describe the structures of these sugar transporters. We have had an eventful year and are looking forward to an exciting year ahead.

been published is our work on a new fluorescent protein from the fish *Sanders vitrious* – which we now call Sandercyanin. The protein purified from the naturally occurring blue walleye (normal walleyes are yellow), and the studies of the protein and its recombinant form allowed us to provide a molecular explanation of adaptation occurring in nature (Ghosh et al., 2016). In order to use the protein for applications in imaging, it is necessary to both improve the brightness of the protein as well as make a monomeric form of the protein. We have now succeeded in engineering a monomeric form that has similar fluorescence properties – our work now is focused on improving the brightness of the protein.

Our work on sialic acid uptake and catabolism in gram negative bacteria has now reached a turning point. The uptake and utilization of Neu5Ac has been shown to have significant pathological consequences to the host. We had initiated a program to carry out structure function studies on these proteins three years ago with funding from a grant from the Department of Biotechnology. The work has now progressed well and the figure above shows the structures of the proteins we have determined. All of these proteins have also been functionally characterized. We have made many interesting findings on individual proteins. The *N-acetyl nueraminic* acid kinase from *H. influenzea, P. multocida, F. nucleatum* and *V. cholera* all are about 80% identical in sequence. Atomic resolution structures determined of these proteins reveals that they are all very similar in structure (less than 1.2Å RMSD) – yet two of the enzymes *(F. nulceatum* and *V. cholera),* shows ordered binding of the two substrates (sugar binding first before ATP binding) while the other two do not. This is confirmed by both isothermal titration calorimetry studies and steady state kinetic studies. The structures of the deaminase proteins from the different bacteria show different oligomeric forms and we are exploring the functional difference arising due to these. Similar interesting fundamental questions are being asked from the structures of each class of proteins. The important achieve the past year has been getting over the bottleneck of producing good diffraction quality crystals – which has resulted in a number of structures.

PUBLICATIONS

1. Banerjee S, Coussens NP, Gallat FX, Sathyanarayanan N, Srikanth J, Yagi KJ, Gray JS, Tobe SS, Stay B, Chavas LMG, Ramaswamy S (2016) Structure of a heterogeneous, glycosylated, lipid-bound, in vivo-grown protein crystal at atomic resolution from the viviparous cockroach Diploptera punctata. ***IUCrJ.*** 3: 282–293

2. Ghosh S, Yu C-L, Ferraro DJ, Sudha S, Pal SK, Schaefer WF, Gibson DT, Ramaswamy S (2016) Blue protein with red fluorescence. ***Proc. Natl. Acad. Sci. USA,*** 113: 11513–11518.

3. Caing-Carlsson R, Goyal P, Sharma A, Ghosh S, Setty TG, North RA, Friemann R, Ramaswamy S (2017) Crystal structure of N-acetylmannosamine kinase from Fusobacterium nucleatum. ***Acta Cryst.*** F73: 356-362.

4. Kumari A, Singh D, Ramaswamy S, Ramanathan G (2017) Structural and functional studies of ferredoxin and oxygenase components of 3-nitrotoluene dioxygenase from Diaphorobacter sp. strain DS2. ***PLoS ONE,*** 12(4): e0176398.

5. Bryce V. Plapp, Baskar Raj Savarimuthu, Daniel J. Ferraro, Jon K. Rubach, Eric N. Brown, Ramaswamy S (2017) Horse Liver Alcohol Dehydrogenase: Zinc Coordination and Catalysis. ***Biochemistry.*** 256 (28): 3632–3646.

6. Ferraro DJ, Okerlund A, Brown EN, Ramaswamy S (2017) One Enzyme many Reactions – Structural basis for the various reactions catalyzed by naphthalene 1,2-dioxygenase. ***IUCrJ,*** 4, 648–656.

7. Budelier MM, Cheng WWL, Bergdoll L, Chen Z-W, Abramson J, Krishnan K, Qian M, Covey DF, Janetka JW, Evers AS (2017) Click Chemistry Reagent for Identification of Sites of Covalent Ligand Incorporation in Integral Membrane Proteins. ***Anal. Chem.,*** 89 (4): 2636–2644.

8. Hoogerheide DP, Noskov SY, Jacobs D, Bergdoll L, Silin V, Worcester DL, Abramson J, Nanda H, Rostovtseva TK, Bezrukov SM (2017) Structural features and lipid binding domain of tubulin on biomimetic mitochondrial membranes. ***Proc. Natl. Acad. Sci. USA*** 114: E3622-E3631.

9. Budelier MM, Cheng WWL, Bergdoll L, Chen Z-W, Janetka JW, Abramson J, Krishnan K, Mydock-McGrane L, Covey DF, Whitelegge JP, and Evers AS (2017) Photoaffinity labeling with cholesterol analogues precisely maps a cholesterol-binding site in voltage-dependent anion channel-1. ***J. Biol. Chem.*** 292: 9294-9304.

8.2

Akash Gulyani

akashg@instem.res.in

DISCOVERIES IN NATURAL LIGHT SENSING, NEURAL REGENERATION AND CELLULAR DYNAMICS

OUR LAB DEVELOPS NEW METHODS TO PROBE BIOLOGY. WE HAVE MADE NEW DISCOVERIES IN NATURAL LIGHT SENSING AND EYE-BRAIN REGENERATION. NEWLY DEVELOPED BIOSENSORS ALLOW US TO VISUALIZE THE ACTIVITY OF CRITICAL SIGNALING AND DISEASE-CAUSING PROTEINS IN LIVE CELLS AND TISSUES.

Visualising signalling dynamics in living cells

Cell signaling is complex, with each critical signaling protein able to control multiple cellular processes and outcomes. However, it is not clear how such precision is achieved. Interestingly, the activity of key signaling proteins is tightly regulated – proteins may be active only in certain locations and for defined time in living cells/tissues. To study this, we have developed new fluorescence-based biosensors to visualize protein activity in live cells and tissues. We first focused on Src family kinases, non-receptor tyrosine kinases that regulate migration, proliferation and overall fate of the cell. Src-kinases influence major diseases including cancer, inflammation and heart diseases. While Src kinases are much studied, it is not clear as to how these kinases control multiple signaling pathways, often simultaneously. Also, the function of individual kinases within the family has been hard to

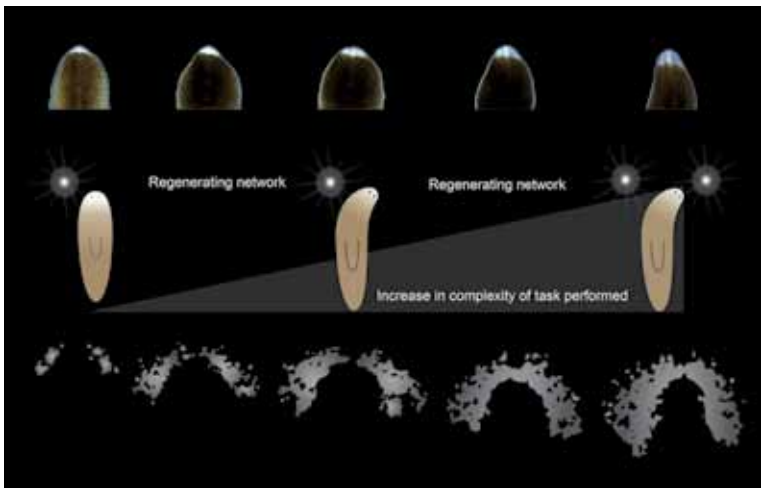


Figure 1: New discoveries in how light is sensed and processed. Schematic showing how planarians with very simple eyes can accomplish complex light sensing and processing, and how regeneration allows us to visualize functional recovery in new ways. We are able to separate 'crude' light sensing from ability to 'compute' and compare; this finding greatly impacts our understanding of how eyes evolved as well as how functional eye and brain regeneration occurs in a living organism.

study due to a lack of tools. Do they work in concert or do they perform exclusive roles? Our work now makes it possible to study Src kinases in 'action', including in disease models.

Our new sensors can specifically report on the activity of individual Src kinases in living cells. To achieve this goal, we have set up a general platform involving high throughput screening and protein engineering to generate sensitive fluorescent reporters that bind and sense active kinases in living cells. Using our new approach, we have now generated sensitive sensors for imaging the activity of Src kinase, Fyn, for the first time. Fyn kinase is a critical player in cancer progression, metastasis and neurodegeneration and these are the first sensors for Fyn activity in cells and tissues. Our biosensor reports that Fyn activity is highly regulated in space and time, peaking at the cell edge in polarized or migrating cells. This information can help explain Fyn's role in regulating cancer metastasis and brain patterning. Also, new biosensor imaging has revealed how Fyn may control multiple cellular/tissue outcomes, due to its ability to directly integrate signaling downstream of different receptor classes at specific sites at the cell membrane. This exciting new result opens new lines of inquiry and can help understand kinase function in complex signaling pathways in both development and disease. We are now collaborating with other researchers to study Src biology in disease and other models. As part of our larger efforts to study cellular dynamics, we have also developed novel probes for mitochondrial imaging in stem cells *(Raja et al 2017).*

Shedding 'light' on neural regeneration

Regeneration of adult neurons and neural networks is a significant challenge. Planarian flatworms show dramatic regeneration ability – it is possible to regenerate an entire organism, including its brain and nervous system, from a small cut piece of an adult worm. Planarians are a powerful model to study regeneration since their nervous architecture is rich, with a cerebral eye, a bi-lobed brain (dorsal ganglion) and a 'peripheral' nervous system including a ventral nerve cord. While regeneration has been examined, relatively little information is available linking neural regeneration to functional recovery.

Planarians possess one of the first examples of an eye and a 'brain' in evolution, and are highly light sensitive. We have made new discoveries that change our understanding of how light in sensed in nature and also been able to link these novel aspects of light sensing to regeneration and functional recovery. We could demonstrate that contrary to previous knowledge, planarians can resolve between very closely related light inputs with only a simple eye *(Shettigar et al 2017).* Our work shows that the planarian cerebral eye and nervous system has the ability to accomplish 'comparative processing' – ability to make small comparisons. Interestingly, during regeneration of the eye and the brain, we can separate this ability of the neural network to 'process' or compute from simple light sensing. This opens a new area of research where we can address the molecular and structural mechanisms underlying neural regeneration and function. We are examining genes and molecules regulating eye and brain regeneration with our collaborator, Dr Dasaradhi Palakodeti. Our new findings allow us to ask how is a simple light sensing eye and brain network is able to 'compute' and compare small differences? This will help regeneration biology (eye and brain) as well as help our understanding of how vision evolved. Towards this end, we have carried out detailed imaging of the planarian brain and the eye in regeneration. Light sensing assays and fluorescence imaging allows us to map eye and neural regeneration in new ways.

PUBLICATIONS

1. Shettigar N, Joshi A, Dalmeida R, Gopalkrishna R, Chakravarthy A, Patnaik S, Mathew M, Palakodeti D, Gulyani A (2017) Hierarchies in light sensing and dynamic interactions between ocular and extraocular sensory networks in a flatworm. ***Science Advances,*** 3(7):e1603025.

2. Raja S O, Sivaraman G, Mukherjee A, Chellappa, A, Gulyani A Facile Synthesis of Highly Sensitive, Red Emitting (2017) Fluorogenic Dye for Microviscosity and Mitochondrial Imaging in Embryonic Stem Cells; ***Chemistry Select,*** 2, 4609-4616.

3. Bansal D, Kulkarni J, Nadahalli K, Lakshmanan V, Krishna S, Sasidharan V, Geo J, Dilipkumar S, Pasricha R, Gulyani A, Raghavan S, Palakodeti D (2017) Cytoplasmic poly (A) binding protein (PABPC2) critically regulates epidermal maintenance and turnover in planarian Schmidtea mediterranea. ***Development*** (In press).

INVITED TALKS

1. Sensing across scales: Imaging cellular dynamics and eye-brain regeneration. Frontiers in Imaging Science, Janelia Research campus, April 2017.

2. New methods for visualizing cell signaling and neural regeneration. University of Copenhagen, June 2017.

3. Discoveries in signaling dynamics, natural light sensing and eye-brain regeneration. University of Calcutta, Kolkata; Meeting on Information transfer across scales- From Molecules to Behaviour, March 2017.

4. New probes and sensors for live cell imaging of cell signaling. Kaleidoscope, a discussion meeting in Chemistry, Goa, July 2017.

8.3

Praveen Vemula

praveenv@instem.res.in

LABORATORY OF SELF-ASSEMBLED BIOMATERIALS: ADVANCED MATERIALS FOR BIOMEDICAL APPLICATIONS

THE VEMULA GROUP DEVELOPS A WIDE RANGE OF SELF-ASSEMBLED BIOMATERIALS TO SOLVE UNMET CLINICAL NEEDS. AT PRESENT HIS LAB FOCUSES ON DEVELOPING INFLAMMATION-RESPONSIVE AND INFLAMMATION-TARGETING HYDROGELS MICROPARTICLES TO LOCALIZED DELIVERY OF DRUGS TO AMELIORATE COLON INFLAMMATORY DISORDERS.

Managing the fluctuating severity of inflammation is critical to the development of effective therapeutics for the treatment of inflammatory diseases. Thus far, my lab has been focused on developing biomaterials to improve the therapeutic efficacy of known drugs to i) enhance the lifetime of transplanted organs while eliminating clinical-limitation, global immunosuppression, and ii) improve the treatment of inflammatory bowel diseases (IBD).

In the history of drug delivery, two breakthrough concepts have revolutionized the way drugs have been delivered. The first breakthrough is the development of excipients to improve

the pharmacokinetics of active drug molecules. The second breakthrough is the development of biomaterials which can release the drugs in a sustained manner (Figure 1). Sustained release of drugs has revolutionized the way chronic diseases are treated by keeping serum concentration of the drug within the therapeutic window for an extended period with a single dose of administration. However, lack of efficient strategy for the treatment of 'fluctuating diseases' is a major limitation in the field. Disease severity is highly variable in fluctuating diseases such as inflammatory/ autoimmune and infectious diseases. Thus, pre-tuning the effective dose of the drug and release kinetics has been a daunting task. Therefore, there are no strategies developed for the effective treatment of fluctuating diseases such as inflammatory/autoimmune diseases. To overcome this limitation in the field of drug delivery; my lab is focusing on developing a novel approach where the severity of the disease controls the release of the drug from biomaterials (Figure 1C & 1D). In this approach, disease-specific biomarker such as an enzyme or cytokine will be identified, and drug-encapsulated biomaterials will be developed which can be stable under normal physiological conditions but will be degraded in response to disease-specific biomarker to release the drug in an on-demand manner in response to the disease severity (Figure 1D).

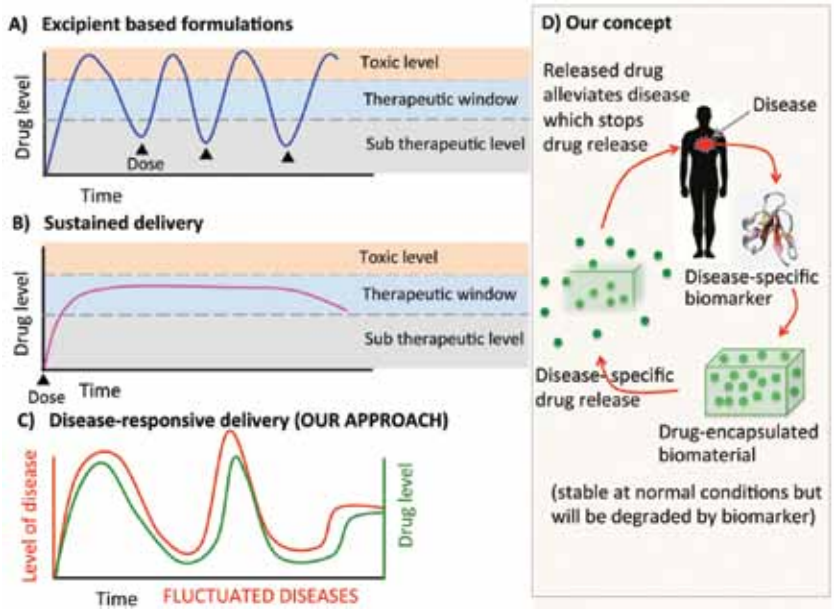


Figure 1:
(A & B) Two breakthrough concepts that revolutionized the drug delivery for medical applications.

(C) Schematic of variable disease severity in fluctuating diseases, and potential drug delivery according to the level of disease.

(D) Our concept of disease-responsive drug delivery for the treatment of inflammatory diseases.

Collaborative Effort

We have been actively collaborating with numerous research groups at inStem, NCBS, and elsewhere national or international labs. A couple of examples of on-going collaborations are described here.

Intra-theme collaboration at inStem

Delivery of transgenes into planaria: The Dasaradhi Palakodeti group is focusing on identifying microRNAs as key regulators in planarian regeneration. They were investigating the role of *miR-124c* in regulating axon guidance, organization of brain

and photoreceptors in regenerating *Planaria.* However, the critical step in studying this process is to deliver nucleic acids (antimiRs, in this instance) into *Planaria,* which is a daunting task. To overcome this limitation, we have developed a novel approach to induce the nucleic acid delivery in regenerating planaria, which enabled identifying a pivotal role of *miR-124c* in the specification organization of neuronal subtypes in the planarian brain (Sasidharan et. al., 2017).

Previously, the Palakodeti group used several modes of delivery of transgenes such as lentiviral vectors, particle bombardment, injection followed by electroporation and transfection using commercial available transfection reagents. None of those methods except particle bombardment using gold particle showed expression of GFP. However, despite early indication, those results were not reproducible.

To overcome these limitations, novel liposomes designed to deliver antimiRs are currently being used to deliver transgenes into *Planaria.* Fluorescent labelled DNA fragments packed into novel liposomes were delivered into intact planarians and also dissociated cells. We observed fluorescence both inside the nucleus and cytoplasm of the cells, both in the intact animal and the dissociated cells. Almost 100% of the transfected cells showed fluorescence indicating efficient delivery of fluorescent labelled DNA. Currently, we are transfecting the expression vectors with planaria-specific promoters driving GFP and mCherry into the intact animals and the dissociated cells to generate planarians expressing GFP.

Inter-theme collaboration at inStem

In utero **delivery of small molecular drugs and antibodies:** Srikala Raghavan's group is investigating the link between macrophage recruitment and ECM disorganization in beta-1 integrin KO embryonic skin. The key step in this investigation is pharmacological inhibition of the inflammatory response by treating KO embryos with NSAIDs, TGF-beta, and NFkB inhibitors. However, selective delivery of these agents to embryos and have a sustained release is critical to eliminate systemic (mother) exposure. Thus, my lab has developed self-assembled particles based biomaterials to deliver these small molecular drugs and antibodies to embryos (Kurbet et al., 2016).

PUBLICATIONS

1. Hiwale AA, Voshavar C, Dharmalingam P, Dhayani A, Muktavaram R, Nadella R, Sunnapu O, Gandhi S, Naidu VGM, Chaudhuri A, Marepally S, Vemula PK (2017) Scaling the effect of hydrophobic chain length on gene transfer properties of di-alkyl, di-hydroxy ethylammonium chloride based cationic amphiphiles. ***RSC Adv.*** 7, 25398-405.

2. Singh R, Hegde B, Baby BV, Sandeep C, Kotla N, Chandrasekar B, Marepally S, Bodduluri H, Vemula PK, Jala VR (2017) Targeted delivery of microbial metabolite, urolithin A protects from chemically (DSS or TNBS) induced colitis in pre-clinical models. ***J. Immunol,*** 198 (1 Supplement), 65-6.

3. Lalitha K, Sridharan V, Maheswari CU, Vemula PK, Nagarajan S (2017) Morphology transition in the helical tubules of supramolecular gels driven by metal ions. ***Chem. Commun.,*** 53, 1538-41.

4. Puroshothaman G, Juvale K, Vemula PK**,** Kirubakaran S, Thiruvenkatam V (2017) Structural studies of 1,2-O-cyclohexylidene-myo-inositol: Insights of hydrogen bonding interactions. ***Acta Crysts. C,*** C73, 20-27.

5. Meka RR, Godeshala S, Marepally S, Thorat K, Rachamalla HKR, Dhayani A, Hiwale AA, Banerjee R, Chaudhuri A, Vemula PK (2016) Asymmetric cationic lipids based non-viral vectors for an efficient nucleic acid delivery. ***RSC Adv.,*** 6, 77841-48.

6. Kurbet AS, Hegde S, Bhattacharjee O, Marepally S, Vemula PK, Raghavan S (2016) Sterile inflammation enhances ECM degradation in integrin β1 KO embryonic skin. ***Cell Rep.*** 16, 3334-47.

7. Divya KP, Miroshnikov M, Dutta D, Vemula PK, Ajayan PM, John G (2016) In situ synthesis of metal nanoparticle embedded hybrid soft nanomaterials. ***Acc. Chem. Res.,*** 49, 1671-80. (An invited review)

8. Vemula PK, Jala VR (2016) Colonic crypts are natural gatekeepers of microbial metabolites to protect stem cells. ***Transl. Cancer Res.*** Vol 5, Supplement 3. (An invited commentary)

9. Wu K, Dhayani A, Slaughter K, Karp JM, Joshi N, Vemula PK, Wang L, Cindass R, Lawson S, Gorantla VS, Davis MR (2016) Graft-implanted tacrolimus-eluting hydrogels prolong survival after vascularized composite allotransplantation. ***Bri. J. Surgery,*** 103, 92.

10. Amit I, et al. Voices of Biotech (2016) ***Nat. Biotechnol.*** 34, 270-275. (Feature)

11. Saha P, Yeoh BS, Singh R, Chandrasekar B, Vemula PK, Haribabu B, Vijay-Kumar M Jala VR (2016) Gut mitochondria conversion of dietary ellagic acid into bioactive phytoceutical urolithin A inhibits heme peroxidases. ***PLoS ONE,*** 1(6): e0156811.

12. Mathiyazhakan M, Upputuri PK, Sivasubramanian K, Dhayani A, Vemula PK, Zou P, Pu K, Yang C, Pramanik M, Xu C (2016) In situ synthesis of gold nanostars within liposomes for controlled drug release and photoacoustic imaging. ***Sci. China Mater.*** 59, 892-900. (COVER FEATURE)

13. Selot R, Marepally S, Vemula PK, Jayandharan GR (2016) Nanoparticle coated viral vectors for gene therapy. ***Curr Biotechnol.*** 5, 44-53.

BOOK CHAPTERS:

1. Mahato M, Sherman NE, Mudnakudu KKM, Joshi N, Briand E, Karp JM, Vemula PK (2017) Prevention of metal exposure: Chelating agents and barrier creams. In: ***Metal Allergy, Springer.***

2. Wang M, Marepally SK, Vemula PK, Xu C (2016) Inorganic nanoparticles for transdermal drug delivery and topical application. In: ***Nanoscience and Dermatology, Elsevier.***

INVITED TALKS

1. Translational science: A tale of four (ad)ventures in science-entrepreneurship! SERB-School of Chemical Ecology, inStem/NCBS, Bangalore, 9th July 2017.

2. Disease-controlled pharmacokinetics: Its applications in biomedical sciences. Research to Industry – Pharmacological insights (workshop), at Acharya & B M Reddy College of Pharmacy, Bangalore 31st March 2017.

3. Disease-responsive nanobiomaterials – an emerging concept in biomedical biomedical sciences. Nano India 2017, Delhi, India, 15-16th March 2017.

4. A tale of three (ad)ventures: A journey of science-entrepreneur. Science Entrepreneur Workshop, SASTRA University, Tanjore, 28th Jan 2017.

5. Clinical translational research: Nanomaterials for biomedical applications. Tibetan Science Conclave IV, Bangalore, 15st Dec 2016.

6. A tale of three (ad)ventures: A journey of science-entrepreneur. Science Entrepreneur Workshop, SASTRA University, Tanjore, 28th Jan 2017.

7. Clinical translational research: Nanomaterials for biomedical applications. Tibetan Science Conclave IV, Bangalore, 15st Dec 2016.

8.4

Dasaradhi Palakodeti

dasaradhip@instem.res.in

DISSECT THE MECHANISM OF TRANSLATION REGULATION CRITICAL FOR REGENERATION AND STEM CELL FUNCTION

MY LABORATORY STUDIES THE ROLE OF TRANSLATION REGULATORS CRITICAL FOR STEM CELL FUNCTION AND REGENERATION. WE USE THE PLANARIAN *SCHMIDTEA MEDITERRANEA,* A ROBUST REGENERATIVE MODEL AND MAMMALIAN EMBRYONIC STEM CELLS TO IDENTIFY REGULATORS CRITICAL FOR TRANSLATION REGULATION THAT DRIVE STEM CELL FUNCTION AND REGENERATION.

Regeneration involves a complex interplay of cellular events tightly coordinated by different layers of gene regulatory networks. While transcriptional, epigenetic and signaling components essential for regeneration are well characterized, the translation regulatory mechanisms critical for regeneration is poorly understood. The work done in my laboratory provides insights into the crucial role of translation regulation in cell state transitions essential for tissue morphogenesis and stem cell function (Figure 1).

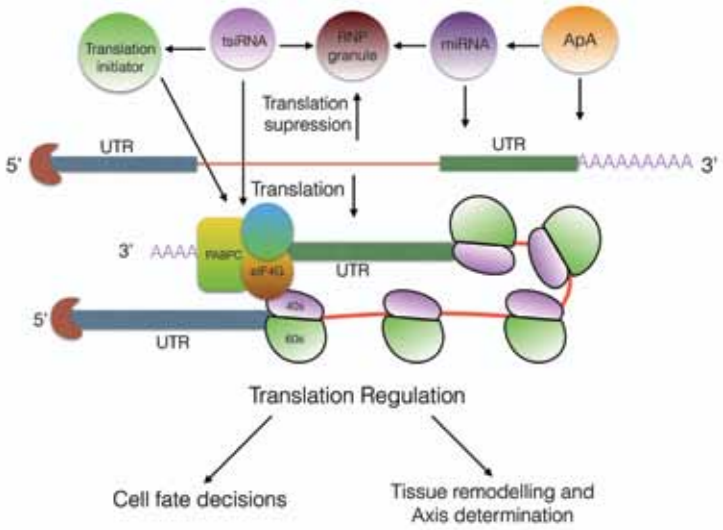


Figure 1: Schematic showing the interplay between different post-transcriptional mechanisms that regulates translation critical for cell fate decisions, tissue remodeling, and axis determination. Arrow marks indicate the cross talk and interactions.

The work addressed key questions in translation regulation, which include:
1. How does the regulation of translation initiation mediated by PolyA binding proteins control cell fate decisions?
2. How does alternate polyadenylation regulate mRNA stability critical for stem cell function?
3. What are the roles of small RNAs in cell fate decisions and tissue organization?
4. How does ribosomal heterogeneity influence cell fate decisions?
Below, I summarize some of the work, which demonstrated the critical role of translation regulatory machinery in stem cell function, lineage commitment and tissue organization during regeneration.

I. Epidermal integrity critically regulated by poly (A) binding protein cytoplasmic (PABPC2) is essential for neoblast function during planarian regeneration. (Bansal et al. 2017)

Identifying the factors that regulate spatial cues critical for coordinating the cellular events is essential for a mechanistic understanding of regeneration. Our work presented here demonstrates the role of epidermis in providing instructive cues essential for neoblast function critical for planarian regeneration. Our study also provides mechanistic insight into the role of poly A binding protein cytoplasmic (PABPC2) in regulating epidermal and extracellular matrix protein (ECM) organization critical for neoblast proliferation near the wound region. PABPCs are RNA binding proteins conserved across eukaryotes, and their role in translational regulation has been extensively studied. Planarian *Schmidtea mediterranea* genome has two PABPC (PABPC1 and PABPC2). Here, we studied novel role for PABPC2 in facilitating the differentiation of neoblast to epidermal lineage essential for the maintenance of epidermal integrity. Furthermore, our study has also shown that the epidermal integrity is critical for the neoblast proliferation near the wound region and blastema formation. Together, the work presented here has broader implications for understanding how the initial events of regeneration such as wound healing and tissue organization could influence the stem cell function critical for tissue regeneration in metazoans.

II. MiR-124 family is critical for Axon Guidance and brain organization during planarian regeneration (Sasidharan et al. 2017)

Our work presented here demonstrates major advances in both the technical

approach and understanding of the critical role of *miR-124 family (miR-124)* in adult neurogenesis, especially in facilitating neural growth, wiring, and organization. *miR-124* is highly conserved and mostly expressed in the central nervous system. *miR-124* in other metazoans facilitates the differentiation of neural stem cell to neurons. However, their role in neural wiring, growth and maturation are not known. We have identified the novel targets of *miR-124* family critical for axon guidance and neural growth, which is essential for proper organization of the brain and photoreceptors. Our results also clearly demonstrated that *miR-124* regulates *slit-1,* required for accurate axon guidance, by modulating the Notch pathway. *This is the first study,* which directly implicates Notch in controlling the expression of axon guidance genes.

Our inability to deliver antimiRs in planaria incapacitated us to perform functional knockdown of microRNAs. To enable the delivery of antimiRs, a novel liposome was developed that showed higher fusogenicity towards planarian cell membrane compared to the commercially available lipofectamine (In collaboration with Praveen Vemula). Our success in developing novel liposomes should also help advance the development of transgenics in planaria. In summary, target prediction and transcriptome sequencing of *miR-124 KD animals* uncovered a novel role for *miR-124 family* in regulating the expression of axon guidance cues and neural growth genes essential for the neural subtype specification, brain and photoreceptors organization in regenerating planaria.

Currently in collaboration with the Akash Gulyani, whose laboratory has recently developed elegant light based assays that monitor the photoreactivity of planarians during regeneration (Shettigar *et al. 2017).* Their work has shown that the planarians can discriminate between different wavelengths of light and predicts that the maturation of neural connectivity to the photoreceptors is essential for discrimination between two different wavelengths of the light. Thus, the light-sensing assay developed by his lab will provide a functional output to dissect the microRNAs that facilitate the neural networks critical for planarian photoreactivity and higher level processing.

PUBLICATIONS

1. Bansal D, Nadahalli K, Laxman V, Krishna S, Sasidharan V, Gulyani A, Raghavan S, Palakodeti D (2017) Cytoplasmic poly (A)-binding protein critically regulates epidermal maintenance and turnover in the planarian *Schmidtea mediterranea.*
In press, ***Development.***

2. Sasidharan V, Marepally S, Elliot S, Baid S, Laxman V, Nayyar N, Bansal D, Sanchez A, Vemula P, Palakodeti D (2017) The miR-124 family of microRNAs is critical for regeneration of the brain and visual system in the planarian Schmidtea mediterranea. In press, ***Development.***

3. Boya R, Yadavalli AD, Nikhat S, Palakodeti D, Pongubala JMR (2017) Developmentally regulated higher-order chromatin interactions orchestrate
B cell fate commitment. In press, ***NAR.***

4. Shettigar N, Joshi A, Dalmeida R, Gopalkrishna R, Chakravarthy A, Patnaik S, Mathew M, Palakodeti D, Gulyani A (2017) Hierarchies in light sensing and dynamic interactions between ocular and extraocular sensory networks in a flatworm. ***Sci Adv.*** 28;3(7):e1603025.

5. Arya D, Sachithanandan SP, Ross C, Palakodeti D, Li S, Krishna S (2017) MiRNA182 regulates percentage of myeloid and erythroid cells in chronic myeloid leukemia. ***Cell Death Dis.,*** 12;8(1):e2547.

6. Lakshmanan V, Bansal D, Kulkarni J, Poduval D, Krishna S, Sasidharan V, Anand P, Seshasayee A, Palakodeti D (2016) Genome-Wide Analysis of Polyadenylation Events in Schmidtea mediterranea. ***G3 (Genes, Genome and genetics).*** 13;6(10):3035-3048.

7. Yim DGR, Krishna S, Lakshmanan V, Koh JLY, Park JE, Cheong JK, Low JL, Lim MJS, Junyu IP, Nah JM, Zhang X, Saj A, an IBH, Iyer NG, Guo H, Sze SK, Raghavan S, Palakodeti D, Dasgupta R. Dynamic expression of tRNA-derived small RNAs define cellular states. (Preprint, http://dx.doi.org/10.1101/1585)

INVITED TALKS

MicroRNAs in planarian regeneration. RNA meeting, held at CCMB from Jan 8th- 10th, 2016.

8.5

Ramkumar Sambasivan

ramkumars@instem.res.in

GENE REGULATORY MECHANISMS UNDERLYING VERTEBRATE DEVELOPMENT

WE STUDY THE NETWORK OF SIGNALING PATHWAYS AND TRANSCRIPTION FACTORS GOVERNING CELL FATE COMMITMENT AND TISSUE PATTERNING IN ORDER TO GAIN MECHANISTIC INSIGHT INTO VERTEBRATE DEVELOPMENT AND EVOLUTION.

The animal body plan is a result of spatial patterning of embryonic tissues to specify development of organs and tissues in the right place in the embryos. The positional cues along the anterior-posterior (A/P) body axis initiate the patterning process. Our work shows the role of these positional cues in generating two distinct groups of muscles in vertebrates.

Head muscles and muscles in the trunk, below neck, represent distinct groups. Whereas trunk muscles aid locomotion, head muscles control the movements of eye, jaw, voice-box etc. and thus, perform a variety of functions such as sensory perception, feeding and vocal expressions. Head muscles also differ from their trunk counterparts by their unique close lineage-relationship with heart. How this diversity in muscle groups along the A/P body axis is generated is poorly understood.

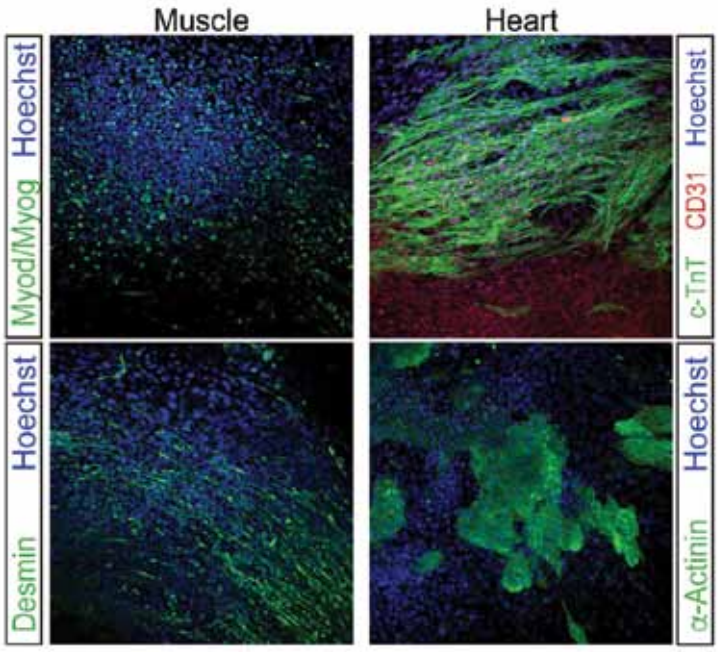


Figure 1: Guided differentiation of mouse embryonic stem cells into muscle and heart. Fluorescence staining of cells cultured in a dish to assess cell type identity. Cells were differentated from mouse embryonic stem cells by a method developed by us based on our discovery of embryonic developmental mechanisms. The staining experiment reveals differentiation of the cells into skeletal muscle based on muscle-specific markers Myod/Myog and Desmin as well as into heart cells by markers cTnT (cardiac Troponin T) and a-actinin. Hoechst is a stain for DNA and marks nuclei of all cells in culture. CD31 marks differentiation into endothelial cells of blood vessels. This approach developed in our laboratory to obtain muscle and heart from stem cells is a significant advance with important clinical value for studying and treating muscle-wasting diseases.

Our work has shown that the divergence in the two muscle groups is established at a very early stage in development. Employing mouse genetics approach as well as embryonic stem cell (ESCs) differentiation experiments, we show that the cues, which establish body axis polarity bifurcate early mesoderm into head and trunk compartments. Mesoderm is the embryonic tissue giving rise to muscles. Wnt and FGF signals are secreted molecules, which function by activating specific gene networks in cells. It is well documented that these two signals act as posterior cues and are central for trunk mesoderm development. In contrast, we reveal that head mesoderm-derived muscle development is independent of Wnt and FGF-driven gene network. Instead, we show that the body axis cues specifying anterior pole of the embryo, i.e., dual inhibition of Wnt and Nodal signals, govern head mesoderm development. Importantly, we show that the dual inhibition-derived mesoderm in the cell culture from ESCs differentiates into heart as well as skeletal muscle. Furthermore, we show that robust head muscle mesoderm development in mouse embryos, requires Wnt inhibition. In essence, we have discovered that the axial polarity cues pattern early mesoderm into head and trunk compartments, which underlies the diversity in the muscle groups generated from these subdomains.

From the stem cell perspective, this work has led to the design of a strategy for generating heart and skeletal muscle from pluripotent cells in culture. While there have been many reports of successful generation of heart from ESCs, derivation of muscle cells has been a challenge until recently. We have developed an efficient strategy to guide the differentiation of ESCs into progenitors, based on developmental cues, that has heart and muscle binary potential (Figure 1). This is the second report of robust

skeletal muscle differentiation from ESCs and the first report of generation of heart/muscle 'bipotent' progenitors. These directed differentiation methods are extremely valuable to model muscle-wasting diseases in cell culture as well as to generate tissue-specific progenitors for therapeutic application.

Our future studies will investigate the mechanism by which Wnt and Nodal dual inhibition activate head muscle and heart mesodermal gene regulatory network. In addition, we are addressing the mechanism underlying the binary fate choice between muscle and heart differentiation. We will also address conservation of the developmental mechanisms that we elucidate for head mesoderm in various animal models, which are evolutionary closely related to vertebrates. This approach will help understand the evolutionary origin of head mesoderm and head muscles and shed light on the extent of conservation of fundamental body axis patterning mechanisms that underlie animal body plan.

PUBLICATIONS

1. Javali A, Misra A, Leonavicius K, Acharya D, Vyas B, Sambasivan R (2017) Co-expression of Tbx6 and Sox2 in an anatomically defined sub-population of neuromesoderm progenitors identifies a transient progenitor cell state. ***Development*** (Accepted)

INVITED TALKS

1. Indian Society of Developmental Biology - Biennial meeting, Pune, 24-27 June 2017.

8.6

Kouichi Hasegawa

kouichih@instem.res.in

DISEASE MODELING AND MECHANISM STUDY WITH PLURIPOTENT STEM CELLS

OUR LAB IS ONE OF INSTEM-ICEMS COLLABORATION LABORATORIES, CONSISTIN OF 2 SISTER LABS; ONE IN INSTEM AND ANOTHER IN ICEMS, KYOTO UNIVERSITY. BOTH ARE JOINTLY STUDYING MOLECULAR MECHANISM OF LIVER AND PANCREATIC DISEASES BY DEVELOPING SUSTAINABLE DISEASE MODELS WITH HUMAN PLURIPOTENT STEM CELLS.

Human pluripotent stem cells (hPSCs) including induced pluripotent stem cells (iPSCs), retain potency to differentiate into almost all cell types in human embryo and adult body, ability to unlimited growth with normal genetics. This hPSC character indicates their highest potential in regenerative medicine, drug development and study of disease mechanisms. Employing hPSCs, we have been conducting development of disease modeling and mechanism study of liver and pancreatic diseases. One of our focuses is malaria *P. vivax* liver stage assay, and another is liver and pancreatic cancer.

Vivax malaria is a global health issue, challenged by undetectable dormant forms in liver responsible for multiple relapses. Lack of suitable models of hepatocytes permissive to *P. vivax*

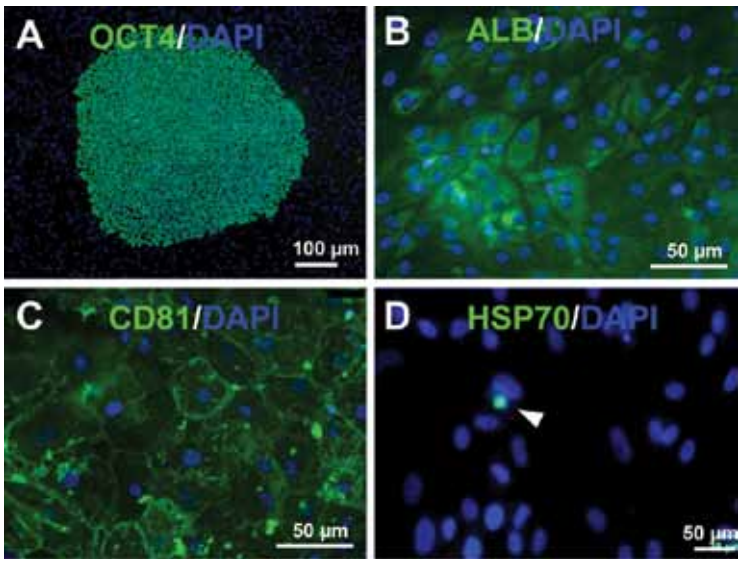


Figure 1: Development of Malaria *P. vivax* liver stage model.

(A) Malaria *P. vivax* patient-derived iPS cell cplony stained with stem cell marker OCT4 immunostaining (green) and nuclear DAPI staining (blue).

(B and C) Patient iPS cell-derived hapatocytes stained with haptocyte marker albumin and Malaria entry molecule CD81 and DAPI.

(D) *P. vivax* Infected haptocytes stained with *P. vivax* - specific HAP70 and DAPI. Arrowhead indicates infected *P. vivax*.

infection is responsible for limited knowledge of infection and relapse mechanisms and unsuccessful efficient drug development. In addition, genetic diversions and variable infectivity, vector susceptibility and latency periods, which is evident across spatiotemporal geographical locations, skews infectivity studies conducted without accounting for its geographical epidemiology. We have developed a robust *in vitro P. vivax* liver-stage assay, employing *P. vivax* patient-derived iPSCs as an unlimited source of hepatocytes and *P. vivax* sporozoites obtained from the same geographical location (Figure 1). For further improvement of our assay, we are currently improving the quality and maturation of the hepatocytes, by utilizing tissue engineering and supplementation of metabolic regents. This assay still would be highly valuable in study of donor-specific drug responses and screening large compound libraries, and would pave way for development of drugs targeting liver-stage malaria.

Pancreatic cancer is the fourth leading cause of lethal cancer with an average five year survival rate of about 7% Live. Cholangiocarcinoma is a rare neoplasm of the bile duct and most patients have a survival of less than 12 months following diagnosis. Both these cancers have a poor prognosis because they are usually detected at an advanced stage. Early diagnosis is difficult because they are usually asymptomatic during initial stages and currently, there is no early-stage biomarker available. To address this issue, we have developed a monoclonal antibody by employing hPSC-derived embryonic liver/pancreatic progenitor cells, which have many similarities with liver/pancreatic cancer cells. The antibody could detect the cell surface of both cancers, while it marked only a small population of healthy ductular endodermal cells. We are identifying the epitope of this antibody, and characterizing the antigen-positive cells. This study may lead to develop regent for early diagnosis and target therapies of the cancers.

PUBLICATIONS

1. Higuchi Y, Nguyen C, Yasuda S, McMillan M, Hasegawa K, Kahn M (2016) Specific Direct Small Molecule p300/β-Catenin Antagonists Maintain Stem Cell Potency. ***Current Molecular Pharmacology,*** 9(3) 272-279.

2. Liu L, Kamei K, Yoshioka M, Nakajima M, Li J, Fujimoto N, Terada S, Tokunaga Y, Koyama Y, Sato H, Hasegawa K, Nakatsuji N, Chen Y (2017) Nano-on-micro fibrous extracellular matrices for scalable expansion of human ES/iPS cells. ***Biomaterials,*** 124, 47-54.

INVITED TALKS

1. Making Malaria The Last Century's Problem, Kyoto University, Japan, April 25, 2016 (Public lecture-iCeMS learning lounge).

2. New State of Pluripotency of Human Pluripotent Stem Cell, International Conference on Science and Technology: Future Challenges and solutions (STFCS-2016), University of Mysore, Mysore, India, August 8-9, 2016.

3. Malaria disease model with patient iPS cells, Joint Retreat on Stem Cell and Differentiation, Shiga, Japan, August 21-22, 2016.

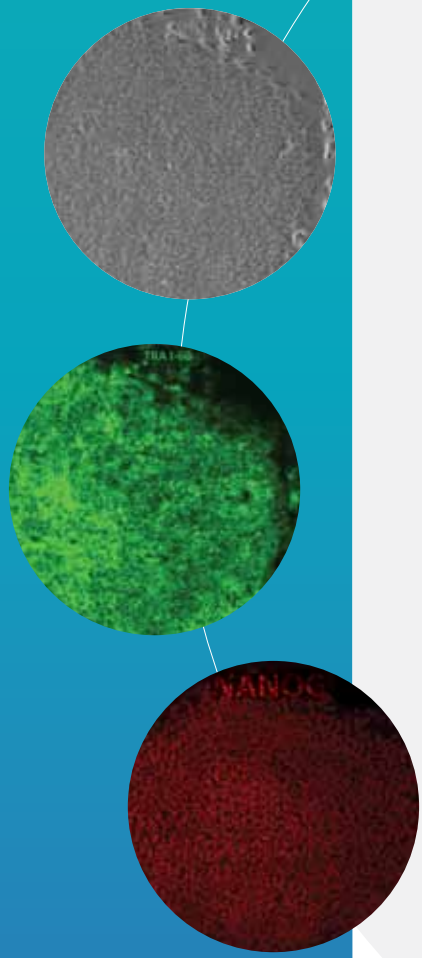
4. Development of sustainable malaria *P. vivax* liver stage mode, Bangalore Life Science Cluster - iCeMS Joint meeting, Bangalore, India, January 23-24, 2017.

9

CSCR

CENTRE FOR STEM CELL RESEARCH, CMC VELLORE

The Centre for Stem Cell Research (www.cscr.in) continues to focus on translational research in cell and gene therapy towards regenerative medicine to bring stem cell science and other novel therapies to management of patients with unmet needs. The concept of teams working on specific themes through multidisciplinary collaborations is being further strengthened to help this goal. Described below are very brief outlines of the specific areas of research at CSCR. More details can be found on the Centre's website.



Thematic research programs

Three major themes for translational research have now clearly evolved:

1. GENE THERAPY

This theme is coordinated by A Srivastava and the group includes R V Shaji, M Murugesan, S Thangavel and S Marepally. The major components of this program involve (a) The AAV vector based gene therapy for haemophilia B (A Srivastava) in collaboration with University of Florida (UF) and Emory University (EmU), USA. Over the last year, this collaboration has progressed to the GMP stage of product development with finalization of a novel transgene in a novel AAV vector and part of its preclinical evaluation. Further testing in the

humanized mouse and non-human primate will be done in the coming weeks. In parallel, based on the prelim data, a pre-IND proposal has been presented to the joint DBT-ICMR Working Group for Gene Therapy as well as submitted to the CDSCO. (b) A major thrust is also on developing a program for gene therapy for the major haemoglobin disorders. Given the continuing success of lentiviral vector approach in multiple clinical trial this being pursued further (R V Shaji) while adding on efforts for genome editing based approach by disrupting the BCL11A gene to increase HbF production – all at the preclinical level at this time. Genome editing approach to gene correction for Wiskott Aldrich Syndrome is being explored (S Thangavel). Genome editing of fetal globin repressors in patient derived hematopoietic stem cells for the treatment of β – hemoglobinopathies and therapeutic genome editing approach for targeted lineage specific expression of FVIII for Hemophilia A is being studied in this program (M Murugesan/S Thangavel). Lipid based gene delivery approaches using bio-inspired cationic amphiphiles are also being explored for gene therapy and genome editing applications. These approaches are directed towards improving safety and efficacy of cationic lipid mediated transfections and developing lipid based nanocarriers to deliver CRISPR/Cas9 tools for efficient genome editing (S Marepally).

2. MUSCULOSKELETAL REGENERATION

The focus of this program is on articular and physeal cartilage replacement, bone and muscle regeneration in different clinical conditions. This group has completed two phase-1 clinical trials for the treatment of large segmental bone defects and physeal arrest in children. Preclinical studies in goats have been conducted for articular cartilage regeneration and osteochondral repair in goat femoral head and rabbits using tissue engineered constructs. In addition, the group is working on a preliminary rat model for sphincter injury. A phase I/II clinical trial has been initiated for the treatment of osteogenesis imperfecta using fetal derived mesenchymal stem cells. Two preclinical studies are in pipeline for osteochondral and segmental bone repair using functionalized scaffolds. Patient recruitment for phase 1 trial for the treatment of large bone defects (gap non-union) in human using hydroxyapatite scaffold loaded with Mesenchymal stem cells has been completed. A total of ten patients have undergone transplantation. All children had shown radiological evidence of union at 2-3 months. One graft failed because of infection 1 year after surgery. In addition to assessment of union with radiographs, CT evaluation at 9 months showed integration at both proximal and distal ends. This area of research is coordinated by V Madhuri and includes several collaborators from within CSCR, CMC, Vellore as well as many external groups within and outside India.

3. APPLICATIONS OF IPSC TECHNOLOGY

Within this theme, there are two areas of translational research. The first is with regard to developing disease models using the iPSC. We aim to create disease models for two inherited red cell disorders, Diamond Blackfan Anemia (DBA) and Congenital Dyserythropoetic Anemia (CDA). We will create mutations (that occur in DBA and CDA) in these genes in a normal induced pluripotent stem cell (iPSC) line by CRISPR/Cas9 mediated gene editing and use the unedited wild type cells as isogenic controls. This would be followed by differentiating these cells to haematopoietic progenitors and then to erythroid cells which would depict the differences in the normal and mutant erythroid cells. Towards this, we have standardised the feeder-free culture conditions for the growth of iPSCs. We have also standardized single cell passaging of iPSCs, which is essential for effective transfection of the gRNAs and

Cas9 used for gene editing. We have explored options to deliver Cas9 and gRNAs to target iPS cells including plasmid mediated or ribonucleoprotein mediated. Currently, we have successfully cloned two gRNAs, each acting upon the following set of genes namely RPS19, RPS24, RPL5 and RPL35a for DBA and SEC23B, CDAN1 for CDA. The following gRNAs, RPS19, RPL35a, SEC23B and CDAN1 have been cloned into the Multiple Lentiviral Expression (MULE) system. In another approach, we have cloned gRNAs against RPL5, RPS24, SEC23B and CDAN1 into LentiCRISPR V2 plasmid, which expresses the Cas9 and gRNA in a single plasmid with puromycin resistance gene for selection. The cloned gRNAs were verified by sequencing and the DNA from the transfected 293T cells showed efficient creation of mutations in these genes by T7 endonuclease assay. The most efficient gRNA targeting each of the genes for DBA and CDA will be used to transfect iPSCs. This work is led by R V Shaji.

The other area of application of the iPSC technology is to develop a bank of iPSCs from healthy donors who have a homozygous HLA haplotype as such individuals have the possibility of being donors for cells/tissues for a large number of individuals in the community. Collaboration with DATRI Stem Cell Donor Registry has been established to help with identifying and contacting the individuals with homozygous HLA haplotype. These individuals will be identified through the DATRI's donor registry from all over the country. Processes of sample collection and transport conditions have been established and initiated. About 120 individuals have been consented, and samples from these have been received at CSCR. Protocols for generation of integration-free feeder-free iPSCs from peripheral blood have been standardized and the developed clones have been characterized for the expression of pluripotency markers. A very efficient cryopreservation protocol has also been developed. iPSC lines from 6 donors with homozygous HLA haplotypes have been generated and they have been tested for the expression of pluripotency markers. More samples will be collected over the next year and 10-20 iPSC cell lines of the most frequent haplotypes in India will be generated. This work is led by D Daniel and R V Shaji with A Srivastava.

4. OTHER PROJECTS

Apart from the major thematic research programs, there are also several areas of project based translational research that scientists at CSCR are pursuing. These includes study of haemoglobin gene regulation in an ex-vivo HSC based human erythropoiesis model using shRNAs and NGS based methods for RNAi screening (R V Shaji), study of MSC derived exosome-based cell-free therapies (Sanjay Kumar), synthesis, design and characterization of biomaterials and scaffolds suitable to control stem cell fate and function, and to engineer human tissues and organs for clinical application (M Ramalingam).

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ALOK SRIVASTAVA

1. Gene therapy futures: Bleeding disorders & Hemoglobinopathies, Blue Ribbon Art and Film Festival 2017 and Rare Diseases Symposium, Bengaluru, March 17-19, 2017.

2. Cure for the major hemoglobin disorders in India, 3rd Global Congress on Sickle Cell Disease, Bhubaneswar, February 21-24, 2017.

3. Cure for Thalassemia major - From allogeneic stem cells to autologous gene correction, New Trends in Hematology, Histiocytic Disorders and Transfusion Medicine Symposium and Workshop, Riyadh (Saudi Arabia), November, 1-3, 2016.

4. Allogeneic Stem Cell Transplantation to Gene Therapy for Thalassemia Major, National Thalassemia Welfare Society, November 2016.

5. Cure for Thalassemia major - From allogeneic stem cells to autologous gene correction, Thalassemia and Sickle Cell Disease Bilateral Workshop (USA-India), Chandigarh, November 5-6, 2016.

6. The Malti Sathe Oration – 2016 - Cure for major hemoglobin disorders in India - The journey so far & the way forward, 57th Annual Conference of Indian Society of Hematology and Blood Transfusion-Haematcon 2016, Jaipur, November 10-13, 2016.

7. Curing Thalassemia major - From allogeneic stem cells to autologous gene correction, 21st Annual Congress of Asia Pacific Blood and Marrow Transplantation Group, Singapore, October 28-30, 2016.

8. Healthcare innovations to address unmet needs - Stem Cells & Gene Therapy, Rotary Club of Madras (India) Chennai, August 9, 2016.

9. The science behind gene therapy in hemophilia, World Federation of Hemophila - 2016 World Congress, Orlando (USA), July 25, 2016.

10. What can we do together to achieve prompt transplant turnarounds for patients undergoing MUD HSCT? 11th International Donor Registry Conference and WMDA Working Group Meetings, Singapore, May 30 – June 2, 2016.

11. Gene Therapy – 'Definitions' of Outcomes: Safety and Efficacy (in the context of hemophilia), International Society on Thrombosis and Haemostasis (ISTH) 2016, Montpellier (France), May 27 2016.

R V SHAJI

1. Disease modelling using induced pluripotent stem cells, Indian Academy of Biomedical Sciences 2017, Bhavnagar, Gujarat, January 6-8, 2017.

2. Applications of induced pluripotent stem cells, National Conference of Young Researchers 2017, Goa University, Goa, March 16-17, 2017.

3. Stem Cells for Haematological Diseases-Disease modelling and Banking for Clinical Applications, GIAN Stem Cell Workshop, University of Hyderabad, Hyderabad, July 21, 2017.

SANJAY KUMAR

1. Mesenchymal stem cells (MSCs) based therapeutic approaches in mice models, DBT-NER Hands on Training Workshop on Stem Cell Biology, ACTREC Mumbai, February 13-18, 2017.

2. Human Mesenchymal Stem Cells (hMSCs) based Therapeutic Approaches in Mice Models, Seminars on Stem Cells and Regenerative Medicine, Anna University , November 11, 2016.

3. Therapeutic application of MSCs in regenerative medicine and in vivo imaging, DBT Live Cell In Vivo Imaging Training Workshop, TRPVB, TANUVAS, Madhavaram, Chennai, November 22-24, 2016.

4. Therapeutic applications of MSCs in regenerative medicine, Expert Talks on Stem Cell Biology, Manipal University, May 29, 2017.

5. Human Mesenchymal stem cells (hMSCs) and approaches to utilise them in regenerative medicine using mice models, Lectures on Stem Cell Biology, Madhav Institute of Technology & Sciences (MITS), Gwalior, March 24, 2017.

SRUJAN KUMAR MAREPALLY

1. Lipid mediated gene therapy: Open questions and future challenges, NIPER-Hyderabad, December 7, 2016.

inStem new building
Courtesy: Ravi Kumar Boyapati, NCBS

10

Academic Programmes

This has been a year of growth and change for our campus, characterized by new connections within India and internationally. We have kicked off multiple post-doctoral fellowship schemes with partner institutions around the world, including the University of Cambridge in the UK, the Max Planck Institute in Germany, Institut Curie in France, and RIKEN in Japan. Our internship programmes for undergraduates are thriving, as we host students from the IITs and IISERs, BITS Pilani, MSU Baroda, and Manipal University. Our continuing engagement with the Tibetan diaspora allows Tibetan students from schools across India to work at labs on campus. Our flagship Chemical Ecology programme attracts students from Northeast India, from six partner institutions including IBSD Sikkim and Rajiv Gandhi University Arunachal Pradesh, to train in laboratories on our campus. The JGEEBILS Examination consortium continues to expand and now includes 17 institutions across India, serving as a central hub for students applying to biology PhD programmes across the country. We were very excited to welcome our alumni from around the world on the occasion of NCBS's 25th year celebrations. Finally, closer to home: we are very excited to welcome our largest incoming group of PhD students.

Connections are also at the heart of student training and mentorship. Our goal is to train the next generation of researchers in the life sciences. This is a field that is rapidly growing and changing. It is impossible to squeeze every known aspect of biology into a standard curriculum, nor would this be desirable since it would be out of date within the year. Instead, we train our students to become perpetual learners, to reach out into areas beyond their comfort zone, and to make connections between disparate facts and phenomena and thereby derive biological insight. Our PhD and Integrated PhD students plunge into science from the day they arrive on campus: they are taught about research ethics and methodology, they rotate in multiple laboratories before choosing which one to join. They are exposed to the latest data and trained to critically evaluate scientific evidence, through a packed calendar of campus seminars and interdisciplinary

journal clubs. They are taught to communicate their science in the Annual Work Seminars and the Sympotein seminar series. They are exposed to state-of-the-art techniques in hands-on workshops such as the Bangalore Microscopy Course. Students in the Masters Program in Wildlife Biology and Conservation have an intensive curriculum that combines on-campus coursework along with work at field sites across India, from the Sikkim Himalayas and the Western Ghats to the Andaman Islands. Through fieldwork these students learn the art of research even as they generate new knowledge to promote the cause of conservation. The end product of this training, mentorship and research is far beyond a thesis: it is a well-rounded researcher, ready to take the next step in an independent research career.

MUKUND THATTAI
Head, Academic Activities

11

SWCCNR

SHANTA WADHWANI CENTRE FOR CARDIAC AND NEURAL RESEARCH

Research at the Shanta Wadhwani Centre for Cardiac and Neural Research (SWCCNR), inStem, has been pivotal in driving exciting new discoveries and efforts in Cardiac and Brain Biology. Unrestricted and flexible support from the Research and Innovation (RIN) division of the Wadhwani Foundation, in synergy with the interdisciplinary collaborative environment that underpins all efforts at inStem, has effectively broadened the scope of impact of the SWCNNR on the campus.

The central aim of research at the Centre for Brain Development and Repair (CBDR) is to understand the cellular, physiological and behavioural basis of brain disorders, and to use this knowledge to test and devise novel therapies. Differentiated neuronal cell types derived from human induced pluripotent stem cells (iPSC), are now poised for the introduction of disease-associated mutations for further studies. Genetically engineered rat models of Autosomal Spectrum Disorders is another unique resource generated at the CBDR. The Centre for Cardiovascular Biology and Disease (CCBD) continues its efforts to identify common cardiac disease mechanisms that can be corrected using small molecules targeted to the contractile protein network at the core functional unit of the human heart. In a recent initiative at the CCBD, next-generation sequencing data of South Asian patients has implicated several non-sarcomeric proteins including receptors in cardiac hypertrophy. The analysis of these is now full-swing employing a combination of cellular models including human iPSC derived cardiomyocytes and mouse genetics. Thus, the programs at the SWCCNR have enhanced both cardiac and neural research capabilities and the translational focus at inStem. In an exciting first for inStem, Minhaj Sirajuddin, a member of the faculty at CCBD, was awarded the EMBO Young Investigator award, the first Indian to be so recognized by this prestigious international award.

Finally, recognizing the importance of generating opportunities, for our youngest researchers to participate in international meetings and visits to top-laboratories world-wide as part of their training, this year, 10 top students/postdoc poster presenters, were selected by a jury of experts for travel awards at the inStem, Annual Meeting 2017. The travel awards, supported by inStem and the Shanta Wadhwani Centre for Cardiac and Neural Research are an invaluable contribution to the overall scientific growth of inStem's early career researchers. We hope that the opportunities offered by this award will highlight their research in the global scientific community and jump-start future academic career paths.

12

RDO@inStem

Research at the Bangalore Life Science Cluster, which includes NCBS, inStem and CCAMP, spans a diverse range of questions and approaches in the broad area of life sciences. The Research Development Office (RDO) was created to facilitate research and training at the Cluster, via research funding. The office continues to offer a concerted mechanism for managing these activities across the three member institutes of the Bangalore Life Science Cluster.

Over the course of the last seven years, the **Sponsored Research** team within the RDO has continued supporting the diverse needs of the campus in fundraising, grants management and contract negotiation for research funding from funding agencies, corporate sources and charitable organizations. More recently, the **Developmental Activities** team at the RDO has started working across with campus colleagues and external individuals and organizations to identify fundraising priorities, facilitate campus funding from philanthropic sources, manage donor engagement events, communications matters and build sustainable relationships with donors.

The Cluster campus was the venue for a learning session titled **"IPI Thematic Session on Philanthropy for Science, Research & Innovation"** for philanthropists, including representatives from the Tata Trust, coordinated by the India Philanthropic Initiative. Noted philanthropists Mr Kris Gopalakrishnan and Dr Kiran Mazumdar Shaw steered the session, which had representation from several Indian research organizations including ICTS-TIFR and TeamIndus, together with philanthropists and Foundation staff.

Developing the portfolio of philanthropic and other support to the campus has required sustained work from the team on all fronts, including our outreach activities. Work at the RDO is made possible by a well-knit group of dynamic and professional individuals, entrepreneurial in spirit and firmly committed to offering several key services to the campus at the boundaries of science, management and outreach.

With a vibrant team, emerging opportunities on the campus and new connections on the outside, we look forward to a rewarding journey further ahead for the RDO, supporting campus research funding and the Endowment Fund.

SAVITA AYYAR

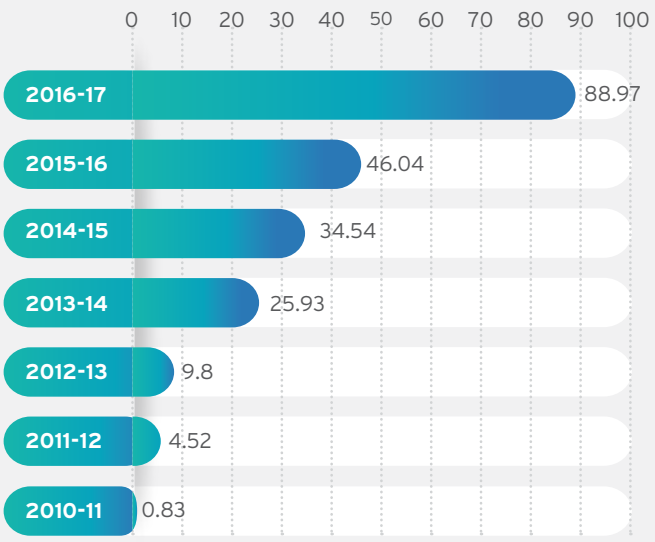

Figure 1: Major Extramural funds received at inStem (in Crore INR)

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inStem International Collaborations


University of Wurzburg, Germany
University of Zurich, Switzerland
IFOM, Italy
IBDM, Marseille
RIKEN, Japan
Kyoto University, Japan

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inStem National Collaborations

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inStem Investigators

APURVA SARIN • Senior Professor & Dean

S RAMASWAMY • Senior Professor

SRIKALA RAGHAVAN • Assistant Investigator

SHRAVANTI RAMPALLI DESHPANDE • Assistant Investigator

AKASH GULYANI • Assistant Investigator

COLIN JAMORA • Associate Investigator*

DASARADHI PALAKODETI • Research Investigator

ARCHANA PURUSHOTHAM • Research Investigator (till March 2017)

PRAVEEN KUMAR VEMULA • Research Investigator

RAVI S MUDDASHETTY • Research Investigator

RAMKUMAR SAMBASIVAN • Research Investigator

TINA MUKHERJEE • Assistant Investigator

MINHAJ SIRAJUDDIN • Assistant Investigator

SUNIL LAXMAN • Assistant Investigator

ARJUN GUHA • Research Investigator

DHANDAPANY PERUNDURAI • Assistant Investigator

VISITING FACULTY

KOUICHI HASEGAWA • Visiting Assistant Investigator (till March 2017)

KENICHI SUZUKI • Visiting Associate Investigator (till March 2017)

JEFF ABRAMSON • (UCLA), Collaborative Science Chair (till March 2017)

JAMES SPUDICH • (Stanford), Collaborative Science Chair

ASHOK VENKITARAMAN • (Cambridge), Collaborative Science Chair

SIDDHARTHAN CHANDRAN • (U Edinburgh), Collaborative Science Chair

PETER KIND • (U Edinburgh), Collaborative Science Chair

MAHENDRA S RAO • (NYIRM, New York), Collaborative Science Chair

SIVARAJ SIVARAMAKRISHNAN • (U of Minnesota), Visiting Faculty

MANEESHA INAMDAR • (JNCASR), Adjunct Faculty

ANIL PRABHAKAR • (IIT, Madras), inStem Associate

SANJEEV JAIN • (NIMHANS, Bangalore), Adjunct Faculty

JYOTSNA DHAWAN • (CCMB, Hyderabad) Visiting Senior Professor

JOHN MERCER • Visiting Faculty (till March 2017)

AZIM SURANI • (Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK) JN Fellowship, DST, India

* In collaboration with IFOM (Milan, Italy)

** In collaboration with iCeMs (Kyoto, Japan)

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inStem Leadership Committees

A. SOCIETY

PROF K VIJAYRAGHAVAN • Secretary to the Government of India, DBT, New Delhi

PROF SATYAJIT MAYOR • Director, NCBS & inStem, Bengaluru

DR ALKA SHARMA • Advisor & Scientist G, DBT, New Delhi

MS GARGI KAUL • JS & FA, DBT, New Delhi

MR CHANDRA PRAKASH GOYAL • Joint Secretary (Administration), DBT, New Delhi

DR SATYAJIT RATH • Scientist, NII, New Delhi

DR KIRAN MAZUMDAR SHAW • CMD, Biocon India Ltd, Bengaluru

DR SUNIL THOMAS CHANDY • Director, CMC, Vellore

PROF H SHARAT CHANDRA • Hon Director, Centre for Human Genetics

PROF ALOK SRIVASTAVA • Head, CSCR & Professor of Medicine, CMC, Vellore

PROF K MUNIYAPPA • Chairman, Department of Biochemistry, IISc, Bengaluru

PROF GOVERDHAN MEHTA • Former Director, IISc and CSIR Bhatnagar Fellow, Bengaluru

PROF P BALARAM • Molecular Biophysics Unit, IISc, Bengaluru

DR CHITTARANJANYAJNIK • KEM Hospital, Pune

DR CHANDRIMA SHAHA • Director, NII, New Delhi

PROF JYOTSNA DHAWAN • Visiting Senior Professor, inStem and Chief Scientist, CCMB, Hyderabad

PROF APURVA SARIN • Dean, inStem, Bengaluru

PROF S RAMASWAMY • inStem, Bengaluru

PROF UPINDER S BHALLA • Dean, NCBS, Bengaluru

MR K KRISHNAMA RAJU • Head – Administration, inStem, Bengaluru

B. GOVERNING COUNCIL

PROF K VIJAYRAGHAVAN • Secretary to the Government of India, DBT, New Delhi

PROF SATYAJIT MAYOR • Director, NCBS & inStem, Bengaluru

DR ALKA SHARMA • Advisor & Scientist G, DBT, New Delhi

MS GARGI KAUL • JS & FA, DBT, New Delhi

MR CHANDRA PRAKASH GOYAL • Joint Secretary (Administration), DBT, New Delhi

DR SATYAJITH RATH • Staff Scientist, NII, New Delhi

PROF CHANDRIMA SHAHA • Director, NII, New Delhi

DR CHITTARANJAN YAJNIK • KEM Hospital, Pune

DR SUNIL THOMAS CHANDY • Director, CMC, Vellore

PROF K MUNIYAPPA • Chairman, Department of Biochemistry, IISc, Bengaluru

PROF ALOK SRIVASTAVA • Head, CSCR and Professor of Medicine, CMC, Vellore

PROF JYOTSNA DHAWAN • Visiting Senior Professor, inStem and Chief Scientist, CCMB, Hyderabad

PROF S RAMASWAMY • inStem, Bengaluru

PROF UPINDER S BHALLA • Dean, NCBS, Bengaluru

PROF APURVA SARIN • Dean, inStem, Bengaluru

MR K KRISHNAMA RAJU • Head – Administration, inStem, Bengaluru

C. SCIENTIFIC ADVISORY COMMITTEE

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PROF SATYAJIT MAYOR • Director, NCBS & inStem

PROF AZIM SURANI • Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, UK

PROF ALEJANDRO SANCHEZ ALVARADO • Howard Hughes Medical Institute, USA

PROF UTPAL BANERJI • University of California, Los Angeles, USA

PROF FRANCESCO BLASI • IFOM (FIRC Institute of Molecular Oncology, Milan), Italy

PROF MARCO FOIANI • IFOM (FIRC Institute of Molecular Oncology, Milan), Italy

DR SATYAJIT RATH • National Institute of Immunology, New Delhi, India

PROF MRIGANKA SUR • Picower Institute for Learning and Memory, Massachusetts Institute of Technology, USA

PROF HELEN SKAER • Emeritus Professor, University of Cambridge

DR MAHENDRA RAO • Senior Scientific Advisor at NYSCF (New York Stem Cell Foundation)

PROF S RAMASWAMY • inStem

PROF JYOTSNA DHAWAN • Visiting Senior Professor, inStem & Chief Scientist, CCMB, Hyderabad

PROF UPINDER S BHALLA • Dean, NCBS

PROF APURVA SARIN • Dean, inStem

D. FINANCE COMMITTEE

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PROF SATYAJIT MAYOR • Director, NCBS & inStem, Bengaluru

DR ALKA SHARMA • Advisor & Scientist G, DBT, New Delhi

MS GARGI KAUL • JS & FA, DBT, New Delhi

PROF S RAMASWAMY • inStem, Bengaluru

PROF APURVA SARIN • Dean, inStem, Bengaluru

PROF UPINDER S BHALLA • Dean, NCBS, Bengaluru

MR K KRISHNAMA RAJU • Head – Administration, inStem, Bengaluru

Non-Academic Staff

E. ADMINISTRATIVE STAFF

K KRISHNAMA RAJU • Head – Administration

KP PANDIAN • Head – Strategy

KM BASAVARAJAPPA • Project Officer

SREENATH BA • Purchase Officer

UMA HR • Assistant Accounts Officer

SHRIKANT BHAT • Senior Project Assistant, Accounts

VALSALA NEYYAN • Administrative Assistant

SHOBHA R • Clerk

SUNITHA R • Project Assistant (Admin)

SHOBHA BN • Project Secretary

F. SCIENTIFIC STAFF

RAJESH R • Engineer C (System Administrator)

ANAND KUMAR V • Engineer C (Electrical)

CHAKRAPANI • Junior System Administrator

SAI SUDHA • Scientist D

DEANISH • Technology Manager

RIFAT NAAZ • Technology Manager

PANKAJ • Technology Manager

AVINASH KUMAR KODICAL • Technology Manager

MUNEESWARAN A • Technical Assistant

G. CONSULTANTS

MAKI MURATA HORI • (till June 2017)

inStem new building

Compilation: Dean's Office
Design: www.thefool.in

Institute for Stem Cell Science and Regenerative Medicine

GKVK - Post, Bellary Road
Bangalore 560065
Karnataka, India

www.instem.res.in