inStem

ANNUAL REPORT 2013-2014

Institute for Stem Cell Science and Regenerative Medicine, Bangalore
(An Autonimous Institute of the Dept. of Biotechnlogy, Govt.of India)

CONTENTS

1. DIRECTOR'S NOTE

inStem now houses six themes and a clinical translational unit. These include the Centre for Inflammation and Tissue Homeostasis (CITH), a joint effort with the IFOM -FIRC Institute for molecular oncology; the Centre for Brain Development and Repair (CBDR), the Centre for Cardiovascular Biology and Disease (CCBD); the Centre for Chemical Biology and Therapeutics (CCBT); the Technology Team (who call themselves the TAS team), and Regulation of Cell Fate. The clinical translational unit run at the Christian Medical College (CMC), the Centre for Stem Cell Research is another endeavor where clinical translation possibilities are being attempted (CSCR, www.cscr.in).

Its time we ask if the culture of 'collaborative inquiry is allowing inStem to tackle major, complex, scientific problems that are difficult to pursue in single investigator laboratories'. One measure is the tangible output that we generate, another is the collaborative culture that must evolve, and the third is the ability to attract people who wish to work in such an environment. I must say that on all accounts, the scorecard gives one reason to hope (if not cheer!) that success is nigh.

Starting with people: we congratulate Azim Surani for being awarded the prestigious Jawaharlal Nehru Fellowship for the year 2014 which he has chosen to house at inStem; warmly welcome Apurva Sarin, formerly at NCBS and now the new Dean at inStem who along with Azim has chosen to initiate a new theme, Regulation of Cell Fate a core thematic of Stem Cell Biology; inaugurate four new research groups at inStem; Tina Mukherjee (Regulation of Cell Fate), interested in understanding how multiple systemic stimuli, intersect with stem cells and their niches; Sunil Laxman (Regulation of Cell Fate), who initiates a programme aimed at investigating mechanisms underlying metabolite sensing and the regulation of cell fates; Arjun Guha (Regulation of Cell Fate) who is interested in the regulation of plasticity of airway epithelial cells in the mammalian respiratory tract during development and following injury repair and

Minhajuddin Sirajuddin (CCBD) whose studies on biophysical properties of core sarcomere proteins will address the consequences of (cardiomyopathy) disease causing mutations to the function of cytoskeletal assemblies.

Collaborations abound at inStem, both within themes and without, here there is a genuine excitement of collaborative discovery evident: just to name a few Colin Jamora's view of homeostasis is expanding to include epigenetics by engaging with Shravanti within the CITH theme. Das from the TAS team is able to envisage the role of miRNAs in almost all aspects of biology starting with neoblast populations in Planaria, to synaptic translational control with Ravi in the CBDR theme. Praveen from the TAS team is keen to get delivery vehicles into the clinical space and is working with the CSCR to take this forward, as well as providing new delivery mechanisms for the CITH theme to look at wound repair.

Tangible output in terms of papers is also beginning and we hope more will be forthcoming. Importantly, our colleagues at CSCR at Vellore are now fully embracing the mandate of clinical translation. The model put in place by Alok Srivastava whose core interests in gene therapy for hemophilia (based on AAV vector in collaboration with Amit Nathwani at UCL, London, UK and Arun Srivastava at UFL, Gainesville, USA) and thalassemia (based on lenti viral vector in collaboration with Trent Spencer at Emory University, USA) have led the way towards the clinic. CSCR is developing a musculoskeletal regeneration programme led by Vrisha Madhuri, and building on the promise of using stem cells, developing a tissue engineering programme aimed at skin and hollow organs. The major challenge at CSCR for its programmes is to define clear translational goals for each activity and develop a team that will help achieve that.

The year has been fruitful in terms of funding for some of our large scale programmes. For example, funding for the programme for studying Autism spectrum disorders at the level of synapses to whole animals, housed at the CBDR theme, and drawing on the skills of our collaborators (Siddharthan Chandran and Peter Kind, as well as Adrian Bird and Richard Morris) from University of Edinburgh and anchored by Shona Chattarji (NCBS) at inStem is now operational, as is funding for CCBT. Excellent catalytic support from the Shanta Wadhwani Foundation for the CBDR and CCBD themes has allowed us to explore daring paths, and we hope to expand our engagement with such enlightened private funding over the next year.

A great challenge ahead lies in inStem negotiating the creation of a unique institutional culture amidst the heady investigator driven environment at NCBS that has been the fertile birthplace for our institute. As our new building, a 200K sq. ft. space comes on line in January 2016, we must ensure that an equally heady atmosphere of science conducted in a thematically collaborative spirit will emerge. This is vital for taking on scientific questions that a single laboratory will find difficult to take on. This is the identity that we hope inStem will differentiate into, distinguishing itself from all other institutes for basic biology.

We hope that as the collective might of the three institutes of the Bangalore Biocluster (NCBS, inStem and CCAMP) come together, the future will be ours to shape.

S. Mayor
Director, inStem

2. ADMINISTRATION REPORT

As we entered the fifth year of inStem, it is heartening to report that the hurdles in construction of the laboratory (delay in transfer of land for building the campus) were all sorted out and construction of the main laboratory block and associated works started in May 2013. During the current year we have completed the other major construction activities – Hostel, Housing and Guest House as well as the Dining Block-cum-sports Complex, and these are being occupied/allotted.

Our science and linkages have been growing. A proposal for funding a programme for studying Autism spectrum disorders at the level of synapses to whole animals, at the CBDR theme was approved during the year. The 3rd floor of the Southern Laboratory Complex (SLC) has been converted into laboratories to accommodate the laboratories of (a) Centre for Chemical Biology and Therapeutics, (b) Centre for Brain Development and Repair and the (c) Macromolecular Form and Function group. With the National Mouse Resource Facility located in the basement of this building, as well as all standard common and major equipments/facilities, [major imaging (including STED), spectroscopy and several high-end equipments and facilities] located in this large laboratory complex, almost all the inStem labs have moved to the SLC. The 2nd floor of SLC has already been functioning as inStem labs. This complements very well with all other major equipments and facilities located in the adjoining buildings [C-CAMP and the old NCBS laboratory block (Eastern Laboratory Block)] providing cutting edge equipments and facilities to the Institute.

During this period our manpower increased to 126, expenditure by 1867 lakh and the quantum of extramural grants from 5673 lakh to 5898 lakh.

inStem also helped organize the All India Cell Biology Conference [December 21-23, 2014] held jointly in our campus and at the Indian Institute of Science.

A MoU was signed with the University of Agricultural Sciences for increased collaboration and sharing of facilities between them and the Cluster Institutions (NCBS, inStem and C-CAMP).

We thank our colleagues in Administration, Scientific and Technical, all of whom have rallied round to support all activities of not only that of inStem which is in constant growth, but of the entire campus, while indeed acknowledging the limitless support of colleagues in NCBS and C-CAMP and the fertile ground provided to this young Institute. The speed and efficiency of the Building team is another highlight which is hastening up the activities of the new Institute, in what is bound to be a remarkable campus both in its physical and intellectual setting.

Mr. T. M. Sahadevan ably assisted this transition period as the Head of Administration and Finance. Search for a new Chief Administrative Officer is in progress and Prof. S. Ramaswamy will take responsibilities of administration and finance, with assistance from Mr. K. Kunhikrishnan. Mr. Sahadevan has taken the major responsibility of making sure the new laboratory complex is completed on time and well for the future development of the Bangalore Life-Science cluster.

T. M. Sahadevan and S. Ramaswamy

3. CENTRE FOR CARDIOVASCULAR BIOLOGY AND DISEASE

Primary cardiomyopathies are heart muscle disorders occurring in the absence of other cardiac conditions, which eventually lead to heart failure. Over 50% of cases are now known to be inherited, with new disease-causing mutations found every week or two. Given that they occur in 1:500 people, they are an enormous public health problem. A primary goal of our project is to understand the condition at multiple scales of organization, allowing us to apply our knowledge to develop small-molecule therapeutics. Our progress in each scale is discussed below.

The first stage of our basic **biochemical and biophysical** assays for tropomyosin mutants has been submitted for publication and is under review. We have been performing fluorescence assays to detect conformational changes caused by calcium of the regulated thin filament, reconstituted in vitro from actin, tropomyosin and the three troponins. In addition to mechanistic heterogeneity we are reporting, we have made an exciting technical observation: the fluorescence assays, only containing actin, tropomyosin, and troponins, correlate well with the results of the far more complex ATPase assays. Put more simply, this may provide us an approximately 30-fold more simple – and therefore more affordable and reproducible-assay for identifying small-molecule inhibitors and activators.

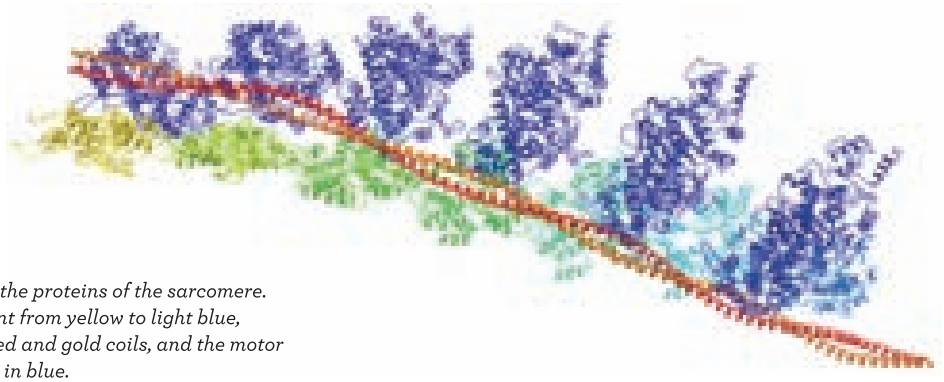
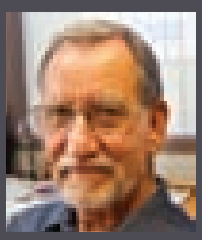
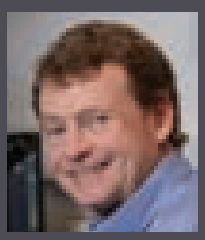


Fig.: Structures of some of the proteins of the sarcomere. Actin is shown in a gradient from yellow to light blue, tropomyosin is shown as red and gold coils, and the motor section of myosin is shown in blue.



JAMES A. SPUDICH
Coordinator



JOHN A. MERCER

For **single-cell analyses** of mutant cardiomyocyte contractility, in collaboration with Maneesha Inamdar of JNCASR we will create the disease-causing mutations in human embryonic stem cells, then differentiate them into beating cardiomyocytes in collaboration with Kouichi Hasegawa. We now have multiple pairs of transcription activator-like effector nucleases (TALENs) that will be used to insert the disease-causing mutations in embryonic stem cells (ESC) and the humanised mice described below. All of our TALEN pairs have been or are being tested in cultured human cells to determine if they cut at the correct site in the genome and do not cut at incorrect sites.

In the **whole-animal approach,** we created a humanised mouse line that expresses the human beta-cardiac myosin (the most commonly mutated protein in inherited cardiomyopathies) using all of the control elements of the mouse alpha-cardiac myosin gene, causing the human myosin to be expressed at high levels in the heart. We built up a breeding stock and begun to inject the TALEN pairs described above into one-cell embryos. We performed a baseline assay with stress and hypertrophy being induced by exercise and drug treatment. To date, the mice expressing the human myosin are indistinguishable from controls.

Our long-term goal is to find common disease mechanisms that can be corrected using small molecules that are targeted to the contractile protein network in a manner that has been illuminated by these studies.

Publications

1. Gupte, T.M., Haque, F., Gangadharan, B., Sunitha, M.S., Rani, D.S., Mukundan, N., Jambekar, A., Thangaraj, K., Sowdhamini, R., Sommese, R.F., Nag, S., Spudich, J.A., Mercer, J.A. (2014) Mechanistic Heterogeneity in Contractile Properties of TPM1 Mutants Associated with Inherited Cardiomyopathies. J Biol Chem, under review.
2. Calliari, A., Farías, J., Puppo, A., Canclini, L., Mercer, J.A., Munroe, D., Sotelo, J.R., & Sotelo-Silveira, J.R. (2014) Myosin Va associates with mRNA in ribonucleoprotein particles present in myelinated peripheral axons and in the central nervous system. Dev Neurobiol 74:382-96.
3. Sotelo, J.R., Canclini, L., Kun, A., Sotelo-Silveira, J.R., Calliari, A., Cal, K., Bresque, M., Dipaolo, A., Farias, J., Mercer, J.A. (2014) Glia to axon RNA transfer. Dev Neurobiol 74:292-302.

Invited Talks

1. Indian Ocean Rim Muscle Colloquium, 12-13 December 2013 NTU – Imperial College London, LKC School of Medicine, Singapore "Molecular mechanisms in heritable sarcomeric cardiomyopathies."
2. 32nd Annual Biomedical Sciences Workshop, 5 August 2014 McLaughlin Research Institute, Great Falls, Montana, USA "Mechanistic heterogeneity in cardiomyopathy-associated tropomyosin (TPM1) mutants."
3. mRNA and Protein Trafficking in Health and Disease, 17-21 November 2013 Weizmann Institute of Science, Rehovot, Israel "Myosin-Va and cell-to-cell RNA transfer."

4. CENTRE FOR BRAIN DEVELOPMENT AND REPAIR

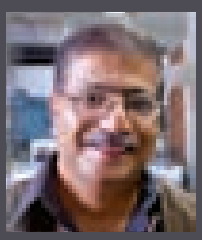
The Center for Brain Development and Repair (CBDR) is an international collaborative center between inStem, NCBS and the University of Edinburgh focusing on neurodevelopmental and neurodegenerative disorders. CBDR received its first instalment of funds from DBT in June of 2014.

To this end, CBDR brings together a range of expertise in several fields of neurobiology including synaptic function and plasticity, human stem cells and cognition-behaviour with the initial goal of investigating autism spectrum disorders (ASDs) and intellectual disability (ASD/ID). As described below, our work over the past year has led to the establishment of three major programmes that combine a range of in vitro and in vivo model systems along with complementary strategies across multiple levels of neural organization.

Siddharthan Chandran, Peter Kind, Sumantra Chattarji

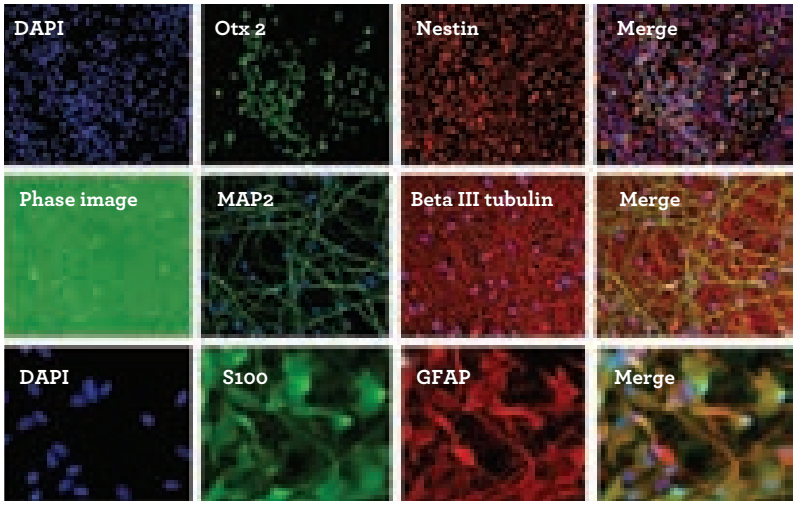
The first programme, led by Prof. Siddharthan Chandran, aims to **model human ASDs "in a dish" to provide new human discovery and testing platforms.** In collaboration with colleagues at Edinburgh, the recently established Neural Stem Cell Facility at CBDR is now streamlining and using human pluripotent stem cell based in vitro systems for both scientific discoveries of cellular and synaptic mechanisms underlying ASD/ID and for subsequent large scale screening for effective pharmaceutical compounds. Standard operating procedures are now in place for the generation and characterization of cortical neurons, neural stem cells and astrocytes derived from human pluripotent stem cells **(Fig. 1)**.

Further, we are now carrying out whole-cell patch-clamp recordings in these cells to probe their electrophysiological properties **(Fig. 2)**. Another key area of investigation, led by Dr. Ravi Muddashetty, centers on the role of microRNAs and microRNA induced silencing complex in



SUMANTRA CHATTARJI
Coordinator

RAVI MUDDASHETTY



< Human pluripotent stem cell derived neural stem cells

< Human pluripotent stem cell derived cortical neurons

< Human pluripotent stem cell derived astrocytes

Fig. 1: Types of brain cells generated in the Neural Stem Cell Facility at CBDR.

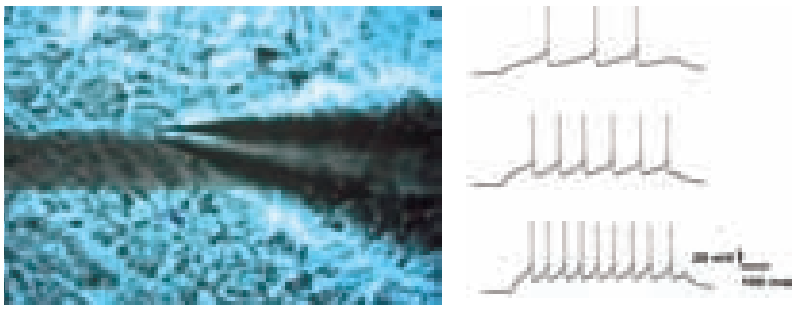
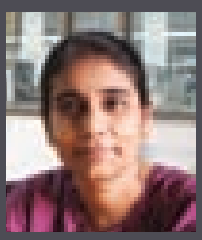


Fig. 2: Left: Image of a patched human pluripotent stem cell-derived cortical neuron.

Right: Action potentials fired by hPSC-derived cortical neuron to depolarizing current steps

the regulation of activity-mediated protein synthesis in neurons, and how these processes go wrong in defective synapses associated with Fragile X Syndrome (FXS) at the level of neuronal differentiation, maturation and plasticity.

In the second programme, led by Prof. Sumantra Chattarji and Prof. Peter Kind, we **examine synaptic and circuit level defects, and their rescue, in autistic networks.** A key priority of this effort is to generate new rat models of highly penetrant single gene causes of ASD/ID to effectively model autistic and cognitive behaviours that can accurately reflect autistic symptoms in humans. These models permit the use of functional MRI in awake, behaving animals that then allows parallel studies in rodent and human using the same modality; allowing, for the first time, human clinical trials to be informed by comparable trials in rodents. We show that a new transgenic rat model for FXS exhibits many of the key physiological and morphological synaptic defects reported earlier



ARCHANA PURUSHOTHAM

SIDDHARTHAN CHANDRAN

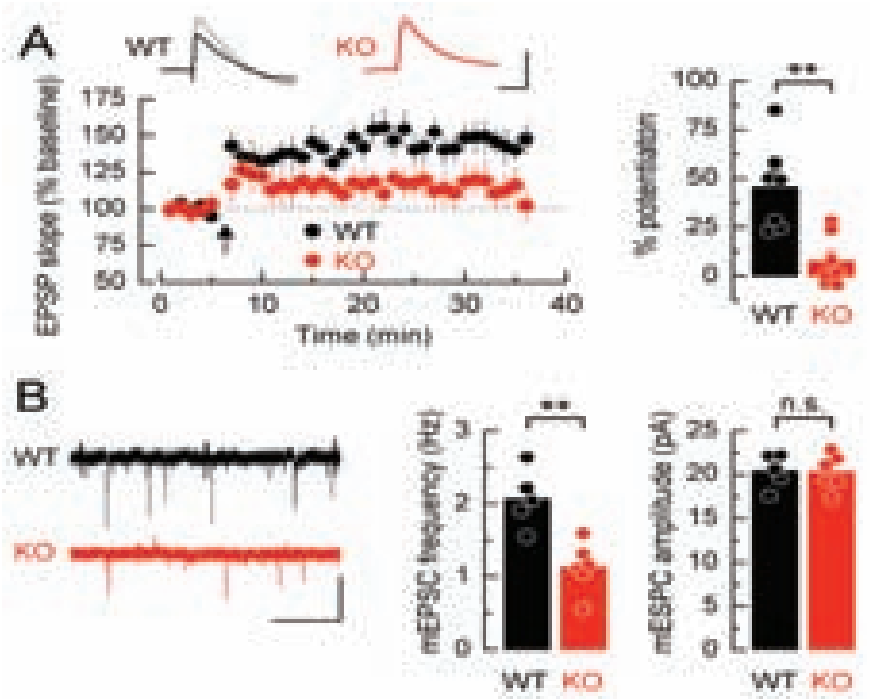


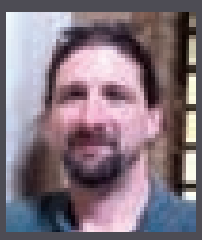
Fig. 3: Abnormal synaptic transmission and plasticity in the amygdala of Fmr1 KO rats.

(A) Time course (left) and magnitude (right) of impaired long-term potentiation in the amygdala of KO rats.

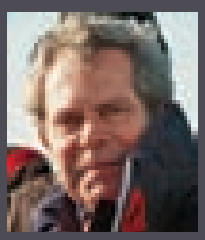
(B) Reduction in mEPSC frequency, but not amplitude, in KO rats. Left: representative traces of synaptic events in KO and WT slices; Right: Summary plot.

in widely used mouse models of FXS. Importantly, the brain-region specific differences in these defects are captured accurately in our new rat model in both the hippocampus and amygdala **(Fig. 3)**, two brain areas that underlie emotional and cognitive dysfunction in FXS. Our findings pave the way for future experiments that will be able to exploit key advantages of the laboratory rat to understand how loss of function Fmr1alleles lead to the complex phenotypes identified in FXS individuals.

We have also identified novel pre-synaptic defects in transmission in the amygdala in mouse and rat models of FXS, which contribute to key emotional symptoms of the disorder. Moreover, some of these synaptic defects are strikingly different from those reported earlier in the hippocampus. With the establishment of primary neuronal cultures from the amygdala **(Fig. 4)**, we are now addressing a wide range of cell intrinsic post-synaptic and pre-synaptic mechanisms, and also analyzing potential points of difference, in terms of molecular and physiological signaling, between the amygdala and hippocampus both in normal and FXS neurons. Finally, using a complementary strategy, we have succeeded in reversing many of the synaptic defects in the amygdala using chronic, in vivo treatment with an mGluR5-antagonist in FXS mice.



PETER KIND



RICHARD MORRIS

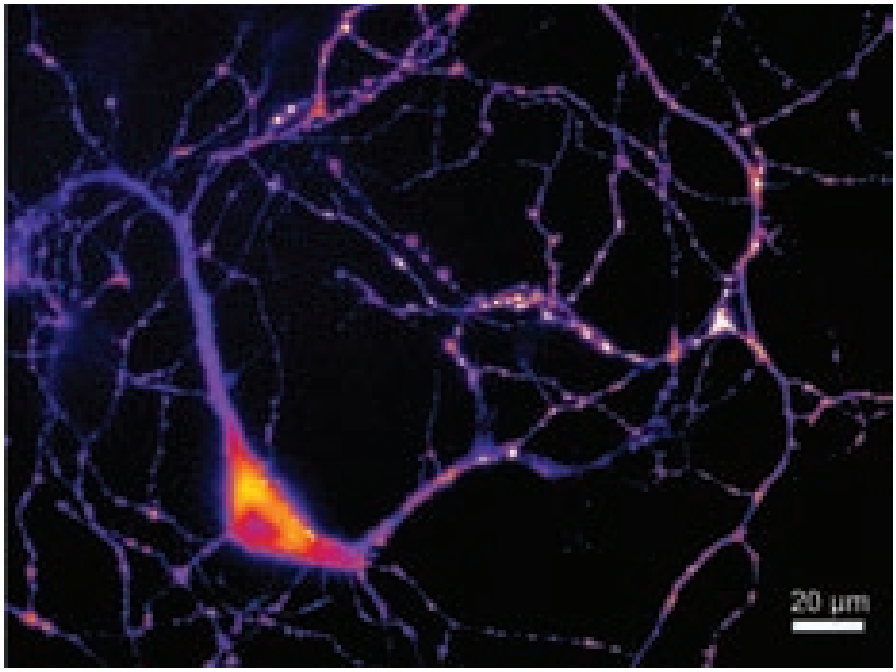


Fig. 4: Amygdala cultured neurons expressing Synaptophysin-PHluorin and stimulated at 10Hz for 30 sec.

The third programme, headed by Prof. Peter Kind and Prof. Sumantra Chattarji, **tests convergence of developmental and cellular phenotypes associated with genetically heterogeneous causes using mouse models of highly penetrant single gene causes of ASD/ID.** The aim here is to determine whether these heterogeneous disorders respond to a small number of tailored treatments throughout the lifespan of the animal and determine whether there are common axes of synaptic neuropathology that can explain a wide range of genetic disorders. Hence, we are investigating the neuropathophysiology associated with Syngap haploinsufficiency, another mechanism underlying ASDs. We gave obtained further evidence for a metabotropic glutamate receptor (mGluR5) signaling axis that is in play and demonstrated convergence of hippocampal pathophysiology in Syngap$^{+/-}$ and Fmr1$^{-/y}$ mice. This line of investigation is being extended through the generation of a new rat model of Syngap.

We are also carrying out behavioural/systems level analysis of the functional consequences of the synaptic defects identified in the above mentioned studies. Using Pavlovian fear conditioning paradigms we have demonstrated that fear learning is impaired in the mouse model of FXS. In parallel, we are also analyzing how fear conditioning is affected in the new rat model of FXS. In a complementary approach, functional MRI analysis of activity in the amygdala and prefrontal cortex

is being carried out after fear conditioning in the FXS rat model. Finally, in addition to studies of fear memory formation and recall, we are investigating social hierarchy and dominance in the FXS rats.

Collaborators: Sanjeev Jain (NIMHANS); Upinder S. Bhalla (NCBS); Matt Nolan (University of Edinburgh) Mike Cousin (University of Edinburgh); Giles Hardingham (University of Edinburgh); Sir Adrian Bird (University of Edinburgh); Sir Ian Wilmut (University of Edinburgh).

Ravi Muddashetty

Neurons 'talk' to each other by electrical and chemical signals through the synapse, 'the site' of their interaction. This 'talk' between neurons is modified and/or intensified by subtle but very specific molecular changes at the synapse. A primary source of this molecular change is activity mediated protein synthesis, which is spatially and temporally regulated to meet local demand and establishes real-time correlation between the signal and the corresponding change. Our lab is interested in deciphering the link between the signal to protein synthesis and its impact on neuronal development and plasticity. We hypothesize that microRNAs and microRNA induced silencing complex (miRISC) form the core regulator of activity-mediated protein synthesis in neurons. This suggests that in neurons, microRNA-mediated inhibition is reversible thereby providing a means for spatiotemporal regulation of protein synthesis, which is critical for synaptic plasticity.

Our work has shown that reversible regulation of translation provides a very specific time window for synaptic protein synthesis in response to neuronal activity. This translation dynamics varies among mRNA subsets (such as PSD-95, Arc, GluR1 and LIMK 1) and contributes to several synaptic events such as surface expression of receptors, cytoskeletal modification and neurotransmitter release. Investigating the change in translation status of these mRNAs in correlation to their association with microRNAs and miRISC has revealed the role RNA binding proteins such as FMRP and MOV10. These proteins act as molecular switches to determine the translation in response to specific neuronal activity. Responding to specific external stimuli such as activation of mGluR or NMDA receptors, FMRP and MOV10 shift mRNAs from inhibitory miRISC to actively translating polyribosomes. This change includes intermediary steps such as changing the composition of RISC, altering the length of poly A and selective degradation of microRNA/mRNAs. We are currently investigating the mechanistic details of these processes. A long-term goal in this regard is to identify the battery of molecular switches that may decode the specific neurotransmitter/growth factor signal to the local protein synthesis at the synapse.

Activity mediated protein synthesis plays an important role in neuronal development and appears to be a major converging point for the defects of neuronal development. In this context we are investigating the role of microRNAs and miRISC in synaptic defects associated with fragile X syndrome at the level of neuronal differentiation, maturation and plasticity. We are in the process of identifying FMRP associated microRNAs in human embryonic stem cells and neuronal precursor cells to study their impact on neuronal and glial differentiation. Using primary neuronal cultures from cortex, hippocampus and amygdala, we are studying the role of FMRP and miRISC on various activity-induced changes in the pre- and post-synaptic compartments and their impact on plasticity. A key aim is to elucidate the molecular determinants of plasticity difference between hippocampus and amygdala with a special interest on the role of FMRP. The focus on FMRP aims to build a model to explain the role of translational modulators at synapse, which would help to elucidate a general mechanism of activity mediated protein synthesis during neuronal development and in developmental disorders.

Archana Purushotham

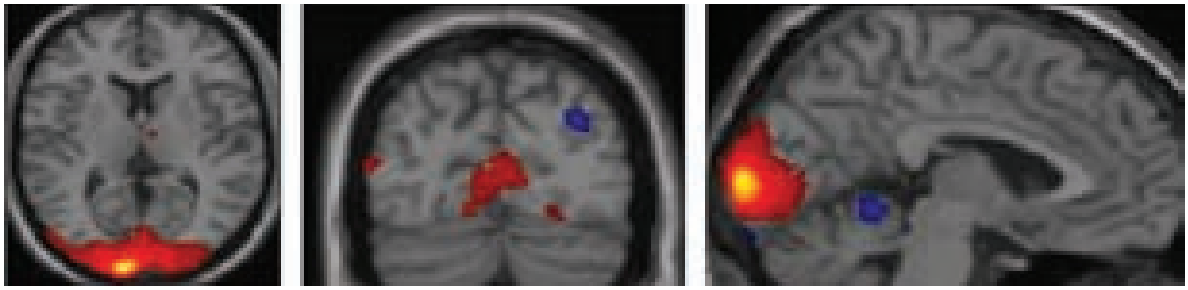
Our lab broadly studies the functioning and physiology of the human brain in health and disease using neuroimaging, and we hope to translate this information into improved diagnosis and treatments for neurological diseases.

COGNITION AND BRAIN CONNECTIVITY

Neural network connectivity in the brain can be studied using MRI. Structural connectivity between different areas of the brain can be studied using diffusion tensor imaging (DTI) while functional Magnetic Resonance Imaging (fMRI) gives us a measure of the functional connectivity.

Normal human individuals differ widely in their cognitive abilities, be it in the language, mathematics, music or emotional intelligence domains. We plan to examine the neural basis of this normal variation by systematically measuring the performance of normal subjects in these and other cognitive domains, and correlating the scores so obtained with structural and functional connectivity strengths between relevant areas of the brain. For example, we would expect that higher scores in language tests would correlate with stronger connectivity between language areas in the brain.

Existent standardized cognitive tests have mostly been developed for, and standardized on, Western populations. We have put together an extensive battery of cognitive tests for Indian subjects, and are in the process of collecting normative data from the Indian population (See http://cogitare.instem.res.in). We have also begun the connectivity imaging study. An example of brain activation in response to visual stimulation in a subject is shown in the image below.



If our hypothesis on the relationship between neural network connectivity and cognitive performance is proved correct, it would be a major step forward in understanding the neural basis of the spectrum of variation in "intelligences" among people.

PILOT STUDY ON OUTCOMES OF ISCHAEMIC STROKE TREATED BY AYURVEDA VERSUS ALLOPATHY

Stroke is the number one cause of major long-tem disability, and its incidence in India is on the increase. An ischaemic stroke occurs when loss of blood flow causes tissue in a part of the brain to die. The only proven Allopathic treatment for a stroke is expensive, time-limited, risky, requires intensive care, but is only partially effective. For various reasons such as accessibility to capable hospitals and affordability, almost no stroke patient in India receives this treatment.

Ayurvedic diagnosis and treatment of stroke does not have a limiting time-window and can be done even in the absence of hi-tech facilities such as intensive care. However, its efficacy or safety has not been formally documented in modern times.

We have started a pilot study of outcomes of stroke patients treated with traditional Ayurveda, compared with those treated supportively with Allopathy. The Ayurvedic patients are being recruited under my Ayurvedic collaborator at a hospital in Puttur. The Allopathic patients are being recruited at the Christian Medical College, Vellore, and we have just obtained ethics approval to begin recruitment there. Patients will be treated according to the respective system of medicine,

and followed for 3 months after their strokes. Extent of recovery and any adverse events will be recorded and compared. Based on the results of this pilot, we would then plan a larger, single-site randomized study to definitively establish the efficacy and safety of Ayurvedic stroke treatment.

Collaborators: Dr. Ravishankar Pervaje, Consultant, Sushruta Ayurveda Hospital, Puttur, Karnataka; Dr. Sanjith Aaron, Professor of Neurology, CMC Vellore, Tamil Nadu; Funded jointly by: Institute of Ayurveda & Integrative Medicine and InStem, Bangalore.

Publications

1. Wijetunge, L.S., Chattarji, S., Wyllie, D.A.J., & Kind, P.C. (2013) Fragile X syndrome: from targets to treatment. Neuropharmacology 68: 83-96.
2. Udagawa, T., Farny, N.G., Jakovcevski, M., Kaphzan, H., Alarcon, J.M., Anilkumar, S., Ivshina, M., Hurt, J.A., Nagaoka, K.A., Nalavadi, V.C., Lorenz, L.J., Bassell, G.J., Akbarian, S., Chattarji, S., Klann, E., and Richter, J.D. (2013) Genetic and Acute CPEB Depletion Ameliorate Fragile X Pathophysiology. Nature Medicine 19, 1473–1477.
3. Barnes, S.A., Wijetunge, L.S., Jackson, A.D., Katsanevaki, D., Osterweil, E.K., Komiyama, N.H., Grant, S.G.N., Bear, M.F., Nägerl, U.V., Kind, P.C. and Wyllie, D.J.A. Convergence of hippocampal pathophysiology in Syngap+/- and Fmr1-/y mice (in review).
4. Purushotham, A., Campbell, B.C.V., Straka, M., Mlynash, M., Olivot, J-M., Bammer, R., Kemp, S.M., Albers, G.W., Lansberg, M.G. (2013) Apparent diffusion coefficient threshold for delineation of ischemic core, International Journal of Stroke (Jun 27. doi: 10.1111/ijs.12068. Epub ahead of print).

Honours and Awards

SUMANTRA CHATTARJI

1. Honorary Professor, School of Clinical Sciences, University of Edinburgh (2014).
2. Fellow, Indian Academy of Science, Bangalore (2014).
3. Chaired the Gordon Research Conference on “The Amygdala in Health and Disease” (2013).

Invited Talks

SUMANTRA CHATTARJI

1. Invited Speaker, International Symposium on "Emotional Memories and Stress: from physiology to psychopathology", Weizmann Institute of Science, Israel (2013).
2. Invited Speaker, Japanese Neuroscience Society, Kyoto, Japan (2013).
3. Chair and Invited Speaker, MCCS-Asia Symposium, Kyoto, Japan (2013).
4. Keynote Lecture, IUPS 2013, The Physiological Society, Birmingham, UK (2013).
5. Keynote Address, Annual Meeting, Pavlovian Society, Austin, TX, USA (2013).
6. Invited speaker, "Long-term potentiation: enhancing neuroscience for 40 years", Royal Society, UK (2013).
7. Invited speaker, Cold Spring Harbor, Asia meeting on "Neural Circuit basis of Behavior and its Disorders", Suzhou, China (2014).

SIDDHARTHAN CHANDRAN

1. Plenary talk, BRAI, NS symposium, Barcelona.
2. TED Global.

PETER KIND

1. Gordon Research Conference on FXS and Autism, NH, USA.
2. FENS Forum, Milan, Italy.
3. Castang Meeting on Neurodevelopmental Disorders2014 GRC FXS and Autism related Disorders.
4. Vice Chair elect for 2016 GRC FXS and Autism related Disorders.
5. 2014 Series of lectures lectures on Brain Development at the Interdisciplinary Institute for Neuroscience, CNRS UMR 5297, Bordeaux 33077, France.
6. 2014 Idex visiting fellow Interdisciplinary Institute for Neuroscience, CNRS UMR 5297, Bordeaux 33077, France.

RAVI MUDDASHETTY

1. International conference on Biotechnology, GGS Indraprastha University, New Delhi- October 2013.
2. Association of Physiologist and Pharmacologist of India conference (APPICON)- NIMHANS, Bangalore- November 2013.
3. Zurich- Bangalore workshop, Bangalore- February 2014.
4. CBDR meeting- University of Edinburgh, Edinburgh, UK- May 2014.

5. CENTRE FOR CHEMICAL BIOLOGY AND THERAPEUTICS

The Centre for Chemical Biology and Therapeutics (CCBT) is an integrated multidisciplinary research centre designed to pioneer new approaches to create small-molecule tools that target novel classes of targets, and use them to conduct hypothesis-generating and hypothesis-driven research on experimental systems of biomedical importance. The CCBTs long-term vision is to foster knowledge-driven therapeutic interventions for important human diseases through fundamental new insights into disease biology, and not to undertake drug discovery per se.

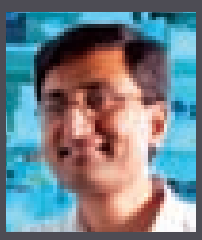
The CCBT's first scientific focus is to explore new approaches for the modulation of intracellular signaling pathways disrupted in disease, by targeting the molecular recognition of key classes of post-transcriptional protein modifications. Success in this work is expected to establish a powerful framework for chemical biology and therapeutics research in the inStem/NCBS campus, to catalyze scientific collaborations with existing programmes, and to promote capacity building at the interface between chemistry and biology.

The CCBT will operate through the uniquely integrated effort of multidisciplinary project teams using common technological platforms, and not as a collection of PI-led labs pursuing independent aspirations subservient to an overall scientific theme. The CCBT represents an inter-institutional collaboration between NCBS and inStem funded directly by the DBT.

Salient achievements

This report comes at the first anniversary of the initiation of work in the CCBT from July 2013. Significant achievements during the past year have been:

- Completion of the fitting-out and equipping of the new CCBT laboratories in the SLC complex at NCBS-inStem. Major equipment including capabilities for high-throughput screening with



ASHOK VENKITARAMAN
Coordinator

liquid-handling robotics, protein biochemistry, and biophysical screening systems has been installed or has been tendered.

- Team leads for Biochemistry/High-Throughput Screening, Computational Chemistry and Molecular Cell Biology have been recruited, and have then recruited teams of post-doctoral and technical staff to initiate research in each area.
- The first scientific programme, whose aim is to develop a palette of chemical tools that exploit different structural mechanisms to modulate the recognition of post-translational protein modifications implicated in intracellular signaling, has been initiated. For 3 novel "undrugged" protein targets, the target protein has been expressed and purified in milligram quantities. High-throughput screening assays have been optimized. A focused chemical library comprising >130K elements has been designed, sourced and organized. Over 1 million in vitro screening reactions have been completed, leading to the identification of >100 active compounds for which validation, computational analysis and analoguing is underway.
- Connections have been developed with CCBT collaborating labs to extend research capabilities in areas of mutual interest aligned to the CCBT's scientific focus. In particular, Professor Sowdhamini's lab (NCBS) uses computational approaches to study the structure of protein-protein interfaces, and has developed multiple computational platforms that will facilitate the discovery of chemical tools modulating these interfaces in collaboration with the CCBT. Professor Yamuna Krishnan's lab (NCBS) creates nucleic-acid based nanomachines as intracellular probes and reporters of biochemical reactions that will enable studies on the chemical biology of intracellular signaling pathways in collaboration with the CCBT.
- Implementation of the foundation arrangements for collaboration with Cambridge University underpinning Ashok Venkitaraman's role as Adjunct Director of the CCBT has been completed.

Agreements with other agencies

Bilateral agreement with Cambridge University, UK to underpin Ashok Venkitaraman's role as Adjunct Director of the CCBT.

Inauguration of the Centre by Professor Sir Leszek Borysiewicz, Vice-Chancellor, University of Cambridge.

Joint meeting of CCBT staff from the Screening team, Computational Chemistry team and Cell Biology team.

6. CENTRE FOR INFLAMMATION AND TISSUE HOMEOSTASIS

The Centre for Inflammation and Tissue Homeostasis (CITH) seeks to make the promise of regenerative medicine a reality and replace/repair tissues lost to disease, trauma, or aging. Currently the theme comprises three full-time faculty members (Drs. Shravanti Rampalli, Srikala Raghavan, Colin Jamora) and a visiting professor from the Genome Institute of Singapore (Ramanuj DasGupta). Research is centered around two foci:

1. The regulation of cells that mediate tissue regeneration and repair;
2. The study of epithelial homeostasis and immune regulation at barrier surfaces. These studies utilize multiple experimental platforms, but a common model system that is studied is skin regeneration and wound healing.

Two exciting research findings illustrate the advances made in the theme over the past year. The first finding was the ability to accelerate wound closure in normal mice and to restore the delayed wound healing found in diabetic mice. Therapeutic application of different compounds were informed by the investigations of the fundamental properties of the wound healing response and the signaling pathways that are perturbed in diseases such as diabetes where the wound closure rate is significantly impaired. The second finding was an unexpected discovery that a protein network that is normally associated with separating the epidermis from the dermis in the skin can induce inflammation when the network is disorganized. This finding opens new avenues of research to understand how inflammation is initiated and, equally important, how this response is terminated to avoid chronic inflammation that is a characteristic of multiple diseases.

Colin Jamora (inStem, IFOM joint research laboratory)

The laboratory is broadly interested in the understanding the molecular mechanisms that govern the wound healing response in the skin. Our ultimate aim is to use these fundamental insights to develop therapies for diseases where the wound healing process perturbed, such as in diabetes.

COLIN JAMORA
(Coordinator)

SRIKALA RAGHAVAN

Over the past year we have made a major investment in investigating the process of scar formation during tissue repair following injury. The scar is comprised of extracellular matrix proteins that provides structural integrity for the damaged tissue and is a scaffold to recruit and stabilize cells that carry out the repair process. When formation of this scar tissue becomes deregulated, it compromises the physiological functions of the organ in a pathological condition known as fibrosis, which contributes to almost 30% of deaths worldwide. This pathological process can affect nearly all tissues and is a prominent characteristic of diseases ranging from cancer to pulmonary, liver, cardiac and kidney fibrosis. Despite the substantial public health burden of fibrosis, surprisingly little is known regarding the molecular and cellular mechanisms that underlie its manifestation. As a result, there are few biomarkers of disease susceptibility and progression or therapeutics to combat its debilitating effects. Unfortunately, the few clinical trials of anti-fibrotic drugs have been disappointing, thus emphasizing the dire need to understand the molecular underpinnings of this disease.

We have found that the transcription factor Snail is upregulated in chronic wounds of the skin and its expression is correlated with various diseases in which fibrosis is present. A transgenic animal engineered to chronically overexpress this protein in epidermal keratinocytes revealed that Snail is sufficient to induce cutaneous inflammation and dermal fibrosis. Surprisingly, we found that Snail activates dermal fibrosis via the exchange of signals between the epidermis and dermis. This novel model contradicts the paradigm in the field that Snail promotes disease by inducing the epidermal keratinocytes themselves to transform into activated fibroblasts in a phenomenon occurring during embryogenesis called an epithelial-mesenchymal transition (EMT). Reconstitution of dermal fibroblast activation by cytokines secreted from Snail expressing keratinocytes in vitro has yielded a set of proteins that heretofore have not been known to play a role in fibrosis. From a basic science perspective, this programme will open up new avenues of research on how the transcription factor Snail mediates intercellular crosstalk between epithelial cells,

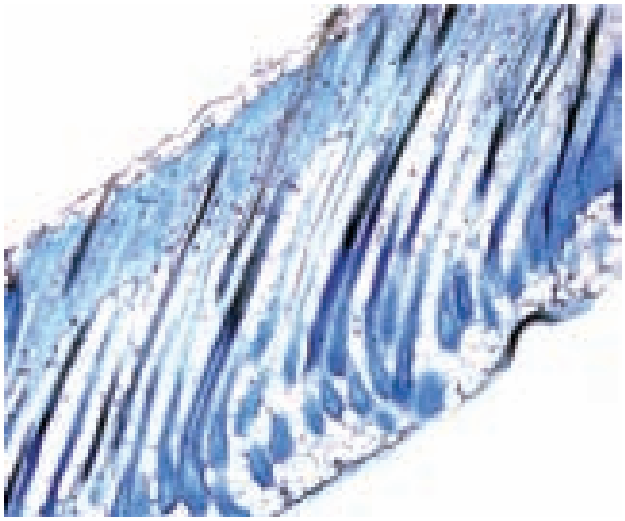
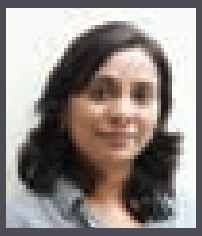


Fig.: Infiltration of mast cells (purple) in the Snail transgenic skin (image courtesy of Neha Pincha).



SHRAVANTI RAMPALLI-DESHPANDE

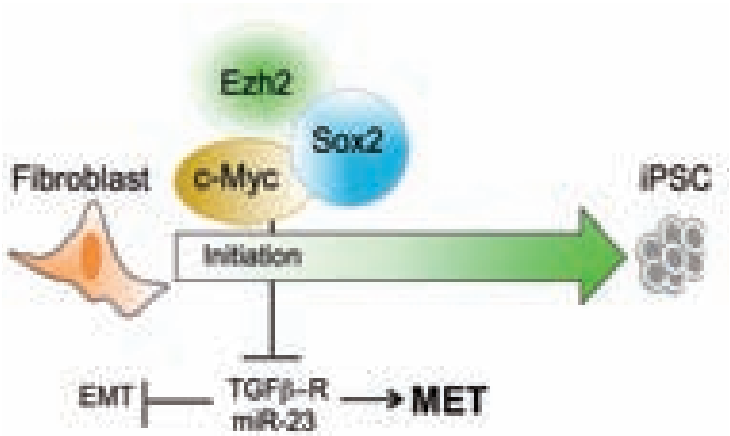
RAMANUJ DASGUPTA

mesenchymal cells, and leukocytes independent of its well-established role in mediating an EMT. The translational benefit of this research programme is the advance in our understanding into the etiology of fibrosis and the potential development of novel therapeutic interventions based on the delineation of novel signaling pathways.

Shravanti Rampalli-Deshpande

Alteration of epigenome by chromatin modifiers plays essential role in numerous biological processes such as development, disease and aging. Studies in animal models and culture-based assays demonstrated critical role of these modifiers in key developmental transitions by regulating cell fate decisions. The main focus of my research group is to dissect the role of histone methyl transferases, a group of chromatin modifiers in regulating the cell fate commitments from somatic and stem cells. Particularly, we utilize human pluripotent stem cells, somatic fibroblasts along with mouse models to address the molecular mechanisms responsible for governing decisions in differentiation, tissue repair and aging. This information is vital in order to understand the pathophysiology of disease and to develop novel therapeutics targeted against chromatin modifiers.

Recently, in an exciting study, we demonstrated that the alteration of chromatin regulator Ezh2 influences the de-differentiation of human fibroblast into pluripotent state. Earlier literature in the cellular reprogramming field has identified the role of individual transcription factors in resetting the chromatin landscape during conversion of fibroblast into pluripotency state, however the engagement with chromatin modulators remained to be elucidated. We found that methyl transferase activity of Ezh2 is required for suppression of pro-EMT signaling during initiation of cellular reprogramming. We showed H3K27me3 activity favors induction of pluripotency by transcriptionally suppressing TGF-β signaling and miR-23a cluster. Unique association of Ezh2 with c-Myc was required to silence aforementioned circuitry. Overall our findings reveal the mechanistic understanding by which Ezh2 restricts the somatic



programme and establish the importance of Ezh2 dependent H3K27me3 activity in transcriptional and miRNA modulation during cellular de-differentiation.

Srikala Raghavan

ROLE OF INTEGRINS IN REGULATING CELL ADHESION, MIGRATION AND EPIDERMAL HOMEOSTASIS

A long-standing goal of our research has been to understand the roles of various keratinocyte integrins in mediating cell-substratum adhesions and its consequences for directed cell migration as well as maintaining cell-cell junctions. As part of this project we have recently started to focus on the role of RNA binding proteins in these processes. This is a collaboration with Dr. Dasarathi Palakodeti's lab. We are also investigating the mechanisms by which integrin β1 and its downstream signaling effectors regulate the assembly and organization of the ECM in the epidermis. Uncovering how integrins regulate ECM assembly and disassembly is highly significant and central to our understanding of normal epithelial homeostasis and will allow us gain insights into their contributions to many disease states such as metastatic skin cancer.

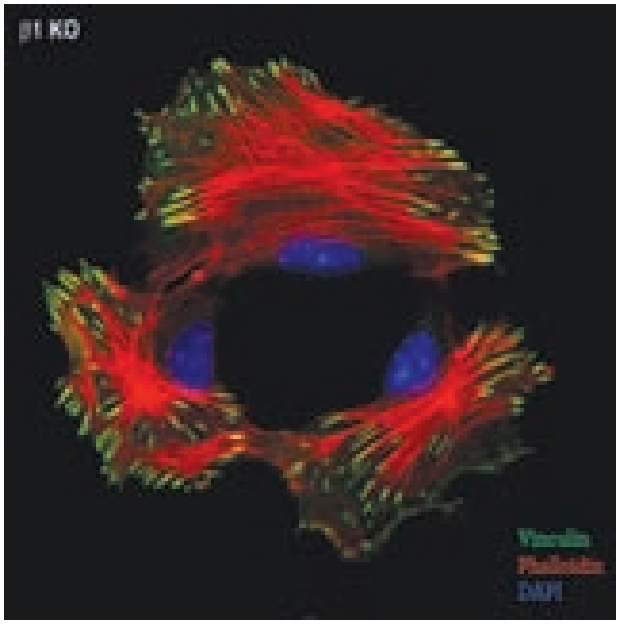
MECHANISM(S) BY WHICH ALTERED TISSUE HOMEOSTASIS INDUCES AN INFLAMMATORY RESPONSE

Loss of β1 integrin in the epidermis results in a strong wound/inflammatory response in the skin. A differentiating feature of this mouse model is the fact that immune/wound-healing response is elicited during embryonic development, at a time when there are no extrinsic wounds and no skin microbiome that may facilitate this process. The integrin KO mouse thus offers and excellent model system to address the nexus between tissue organization and signals mediating an immune response that may be involved in the process of scar less wound healing in embryos.

ROLE OF SMALL RNAS IN EPITHELIAL STEM CELLS

The regulation of gene expression in murine epidermal/follicular stem cells that control their ability to regenerate or differentiate into multiple cell lineages of the adult skin has been an area of active research in the past few years. While expression profiling of purified stem cells have revealed interesting insights into the genes that may be responsible for maintaining pluripotency or activating stem cells along differentiation lineages, much less is known about how these gene may be coordinately regulated in the stem cell niche. The recent discovery of microRNAs (miRs) as global regulators of gene expression make them ideally suited to be involved in modulating the

activity/expression of a network of genes involved in stem cell maintenance and differentiation. Our lab is working in close collaboration with the DasGupta lab (at the Genome Institute, Singapore) and Dr. Dasarathi Palakodeti's lab (TAS theme) to investigate the function of microRNAs (and their putative target genes) as well as tRNA derived small RNAs TRFs in the regulation of epidermal stem cell self-renewal and differentiation, and their interactions with the stem cell niche, with a particular focus on factors that regulate the ECM at the stem cell niche.



Ramanuj DasGupta

MOLECULAR REGULATION OF STEM CELL POTENCY BY tsRNAS

Collaborators: DasGupta, Palakodeti and Raghavan Laboratories.

Small RNA mediated regulation of gene expression has gained utmost importance because of their regulatory role in diverse cellular processes, and their ability to influence changes in global gene expression both in normal development and tumorigenesis. Our initial sequencing studies aimed at identifying differentially expressed small RNAs between stem and non-stem (transit amplifying or differentiating) cell populations have identified a novel class of small RNAs, called tsRNAs that are specifically absent in stem cells but are highly upregulated in differentiating cells. Interestingly we made similar observations in both mouse embryonic stem cells (mESCs) as well as murine hair follicle stem cells suggesting a conserved regulation/function of tsRNAs in stem cell regulation. Our preliminary studies using misexpression of tsRNAs in mESCs suggest that tsRNAs may be involved in promoting differentiation of mESCs. We aim to:

1. Investigate the physiological and molecular function of tsRNAs in the regulation of self-renewal versus differentiation;
2. Determine sub-cellular localization and expression of tsRNAs in stem versus differentiated cells (both in mESCs, and adult hair follicle SCs);
3. Identify the molecular mechanisms involved in the biogenesis and processing of tsRNAs.

GENERATION OF NOVEL PATIENT-DERIVED 3-D CANCER MODELS FOR PREDICTIVE TOXICOLOGY AND HTS/HCS-BASED SYNTHETIC LETHAL SCREENS

Collaborators: DasGupta, and Vemula Laboratories.

Current high-throughput/high-content screening (HTS/HCS) platforms mainly make use of 2-D cell culture models. However, while cells grown in 2-D culture systems are easy to handle and maintain, they do not recapitulate native biological microenvironment especially in terms of cell-cell, cell-matrix interactions and differentiation. Therefore the current screening platforms are not viable structural and functional representations of tissue morphology and function in vivo. On the contrary, though animal models act as a good model system for testing efficacy and/or toxicity of small molecules/drug leads, screening a large number of library compounds using animals is cost-intensive and raises ethical issues. The DasGupta lab at GIS-A*STAR and the Vemula lab are therefore engaged in developing reliable, predictive 3-D screeing models as an alternative to animal screening. Specific patient-derived 3-D models being developed include human colorectal carcinoma (CRC), non-small cell lung carcinoma (NSCLC), and oral squamous cell carcinomas (OSCCs).

Publications

1. Lee, D.J., Chen, S.W., Du, F., Nakasaki, M., Shih, V.F.S., Hoffmann, A. and Jamora, C. (2014) Regulation and function of the caspase-1 inflammasome in a wound-like microenvironment. J. Investigative Dermatology. (in press)
2. Aung, A., Seo, T., Lu, S., Wang, Y., Jamora, C.*, del Alamo, J.C.* and Varghese, S.* (2014) 3-D Traction stresses activate protease-dependent invasion of cancer cells. Biophys. J (in press). * co-corresponding authors
3. Rao, R.*, Dhele*, N., Cheemadan, S., Ketkar, A., Rao, J.G., Palakodeti, D. and Rampalli, S. Ezh2 mediated H3K27me3 activity facilitates somatic transition during pluripotent reprogramming. (Communicated)
4. Kaur, R., Morrison, L., Aiken, C., Rao, R., Del Bigio, M., Rampalli, S. and Werbowetski-Ogilvie, T. (2014) Neural derivatives from hESC model cellular and molecular events contributing to paediatric brian tumorigenesis. (In Review)
5. Brahme, N.N., Harburger, D.S., Kemp-O'Brien, K., Stewart, R., Raghavan, S., Parsons, M., Calderwood, D.A. (2013). Kindlin binds migfilin tandem LIM domains and regulates migfilin focal adhesion localization and recruitment dynamics. J Biol Chem. Dec 6;288(49):35604-16. Epub 2013 Oct 28.
6. Hegde, S., and Raghavan, S. (2013). A skin depth analysis of integrins: Role of the integrin network in health and disease. Cell Commun Adhes, 20(6): 155-69.

7. Huet-Calderwood, C., Brahme, N.N., Kumar, N., Stiegler, A.L., Raghavan, S., Boggon, T.J, Calderwood, D.A. (2014) Differential binding to the ILK complex determines kindlin isoform adhesion localization and integrin activation. J Cell Sci. Aug 1. pii: jcs.155879. [Epub ahead of print] PMID: 25086068.

Honours and Awards

JAMORA

1. Scientific Advisor, Aeon Biotherapeutics (Taiwan, ROC). 2014 – present.

Invited Talks

JAMORA

1. Joint IFOM-MBI Conference: Mechanobiology and Cancer. Milan, Italy. "Investigating the role of fibulin-5 in linking tissue stiffness and inflammation." July 2014.
2. National Taiwan University Medical School, Deparment of Orthopedic Surgery. Taiwan, China 2014. "Cellular and Molecular Mechanisms of the Wound Healing Response".
3. National Taiwan University, Department of Chemical Engineering. Taiwan, China 2014. "What can wound-healing teach us about cancer?".
4. Cellular and Molecular Mechanisms of Disease Processes. Kashmir, India 2014 (keynote presentation declined).
5. NCBS Annual Meeting. Bangalore, India 2014. "Regulation of inflammation in the wound-healing programme by epidermal keratinocytes".
6. University of Dundee-NCBS-inStem, Bangalore Symposium 2013. "Intercellular signaling networks in tissue scarring and fibrosis".
7. Unilever-NCBS Symposium. Bangalore, India 2013. "Wound healing and related diseases".

RAMPALLI

1. Invited speaker "Role of Ezh2 in pluripotent reprogramming" at the Indian society for developmental biology meeting (InSDB). TIFR Mumbai, 1-4th December 2013.
2. Invited speaker, "Role of Ezh2 in pluripotent reprogramming" at the AICBC meeting. Bangalore, 21-24th December 2013.

RAGHAVAN

1. McLaughlin Research Institute, Montana, August 2013.
2. All India Cell Biology Conference, Bangalore December 2013.
3. Annual Talks, NCBS Bangalore, January 2014.
4. Singapore Skin Meeting, Singapore, March 2014.
5. Cutaneous Biology Meeting, Brisbane, Australia, September 2014.

DASGUPTA

1. Invited speaker, Dept. of Biochemistry, SUNY, Buffalo, NY, Oct 2013.
2. Invited speaker, Bioinformatics Institute, A*STAR, Singapore, Apr 2014.
3. Invited speaker, Developmental Biology, VIB, Brussels, Jun 2014.
4. Invited speaker, Neurobiology, ULB Brussels, Jun2014.
5. Keynote speaker, Enabling Genomic Technologies in Asia, Singapore, Sept 2014.
6. Invited speaker, Cutaneous Biology meeting, Queensland, Australia, Sept 2014.

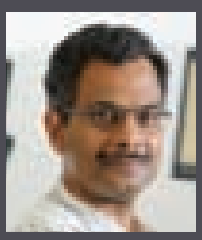
7. TECHNOLOGIES FOR THE ADVANCEMENT OF SCIENCE

InStem core mandate is to carry out theme-based research in a collaborative interdisciplinary fashion on problems that are important and difficult to answer as a single PI-driven initiative. Although individuals are champions in one particular technique, often one needs a larger arsenal of tools to solve multifaceted scientific problems. To cultivate this culture, the Technologies of Advancement of Science (TAS) theme brings a group of individual PIs who work together to develop technologies that not only advance their long-term scientific questions, but also enables the entire scientific community to enhance the level of science being done here. The idea of using techniques as tools sounds good, but mastering modern scientific techniques like next-generation sequencing, mass-spectrometry, etc., takes years of training. The TAS theme strives to develop new technologies and nurture existing-technologies by collaborating across themes and the campus.

Ramaswamy S

The laboratory's main focus is studying structure-function relations of biomolecules including a variety of biophysical approaches. There are several projects in the lab that range from understanding molecular signaling underlying stemness and differentiation to infectious diseases. The laboratory also has a well-defined focus towards technology development. Two ongoing projects are described in this report.

Gram-negative bacteria such as **Haemophilus influenza, Fusobacterium nucleatum, Pasteurella multocida** and **Vibrio cholera** cause several diseases in humans and animals. These bacteria scavenge Sialic Acid (SA) from the host and decorate it as the outermost sugar on their LPS/LOS, which mimic host cell oligosaccharides. This is an example of molecular mimicry that allows pathogenic bacteria to evade the host immune system and influences biofilm formation (oral mucosa) leading to sepsis. SA is transported into bacteria by periplasmic binding proteins that belong to TRAP transport system. We solved the crystal structure of periplasmic binding proteins

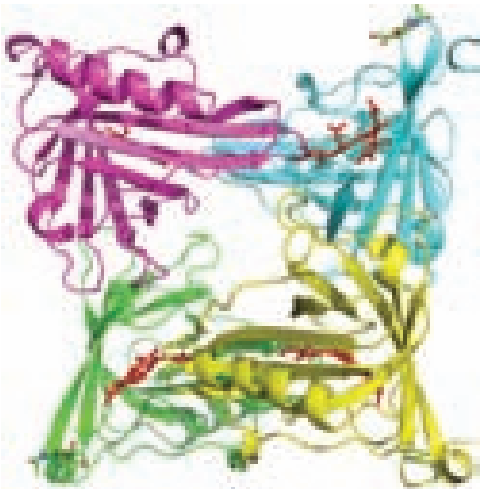


RAMASWAMY S.
Coordinator

AKASH GULYANI

from three pathogenic bacteria. We also analyzed the binding site of sugar at the residue level. The structure and thermodynamics analysis suggest that the periplasmic binding proteins have a very well conserved binding pocket and similar binding affinities to SA.

The discovery and development of fluorescent proteins in the near-infrared spectrum have revolutionized many areas of molecular and cell biology. We have recently discovered Sandercyanin fluorescent protein (SFP) from Blue fish (Sander vitreus) that shows interesting fluorescent properties – including a 300 nm Stoke shift and infrared emission. The crystal structure of the wild type protein shows that it is a tetramer. We have engineered mutations to make a monomer with very similar fluorescent properties. Recently, we have solved the X-ray crystal structures of recombinant holo-SFP and apo-SFP forms of wild type and its monomer mutants and



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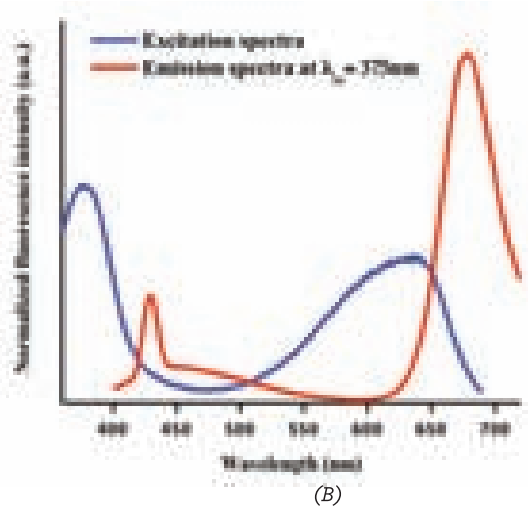

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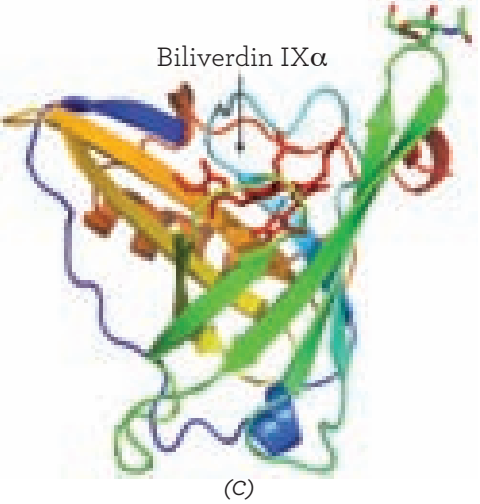
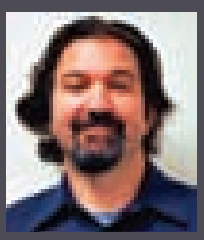


(C)

Fig.: Showing the X-ray crystal structure
A. Wildtype SFP
B. Overlap of excitation and fluorescence spectra of Sandercyanin.
C. Monomer SFP

DASARADHI PALAKODETI



JEFF ABRAMSON

seen significant changes in the conformation of amino acids and biliverdin in the ligand-binding pocket. Sandercyanin has advantages over the existing fluorescent protein in many ways. Hence, we aim to develop Sandercyanin as a potential marker for in-vivo imaging to understand different biological processes.

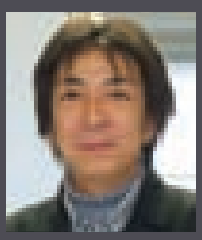
Two projects that we hope to make significant progress in the coming year include the understanding of some of the genomic properties of stem-like cells isolated from buccal mucosal cancer tissue and the role of glycans in planarian regeneration.

Praveen Vemula

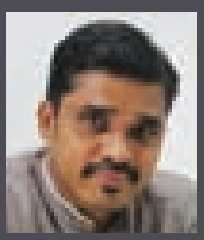
Core interest of my lab is translational research by developing novel biomaterials and concepts to solve unmet clinical needs. Delivering drugs to patients in a safe, effective, and compliant manner is a major challenge for the treatment of many diseases, inflammatory and autoimmune diseases in particular in which severity of the disease is highly fluctuated. Conventional polymeric-drug-delivery systems produce a sharp initial increase in concentration to a peak above the therapeutic range, followed by a fast decrease in concentration to sub-therapeutic levels. **The Holy Grail of drug delivery is an autonomous system that titrates the amount of drug released in response to a biological marker of disease activity, ensuring the drug is available at a therapeutically relevant concentration only when needed.** Such a system must rapidly release drug in response to fluctuations in severity of disease, patient-to-patient variability, and local environmental factors. Inflammatory diseases, like arthritis and organ transplantation-induced immune response, are ideal opportunities to test these systems, as symptoms vary over time in direct proportion to the immune system activation and the local enzymatic milieu of the inflamed tissue can be exploited in the design of the delivery system.

Our lab long-term research goal is to develop disease-responsive drug delivery systems to make a paradigm change in the treatment of inflammatory diseases. As a first-goal, **we have focused on developing injectable immunosuppressant-laden hydrogels to prevent rejection of vascularized composite allografts.**

Following a tissue graft transplant – such as that of the face, hand, arm, or leg, also known as Vascularized Composite Allotransplantation (VCA) – it is standard for doctors to immediately give transplant recipients immunosuppressant drugs to prevent their body's immune system from



KOUICHI HASEGAWA



PRAVEEN KUMAR VEMULA

rejecting and attacking the new body part. However, there are toxicities associated with delivering these drugs systemically, as well as side effects since suppressing the immune system can make a patient vulnerable to infection.

Our lab in collaboration with Robert Rieben (University Hospital of Bern, Switzerland) and Jeffrey Karp (Harvard Medical School, USA) has developed a hydrogel loaded with the immunosuppressant drug tacrolimus. This injectable self-assembled hydrogel releases the immunosuppressant-drug, tacrolimus, only in response to proteolytic enzymes that are overexpressed during inflammation and can overcome existing limitations in VCA maintenance therapy. The hydrogel-drug combo is injected under the skin after transplant surgery. The hydrogel remains inactive until it detects an inflammation/immune response from the transplant site, at which point it delivers the immunosuppressant drug for months locally within the transplanted graft.

To validate this approach the hind limb of Brown Norway rats were transplanted to Lewis rats. In pre-clinical studies conducted by our team, a one-time, local injection of the hydrogel-drug combo prevented graft rejection for more than 100 days compared to 33.5 days for recipients receiving only tacrolimus and 11 days for recipients without treatment or only receiving hydrogel. In addition to extending the lifetime of the transplanted limb, the current approach also completely eliminated the systemic toxicity of immunosuppressant. In the future, we plan to expand this approach for the treatment of numerous diseases such as rheumatoid arthritis and inflammatory bowel disease.

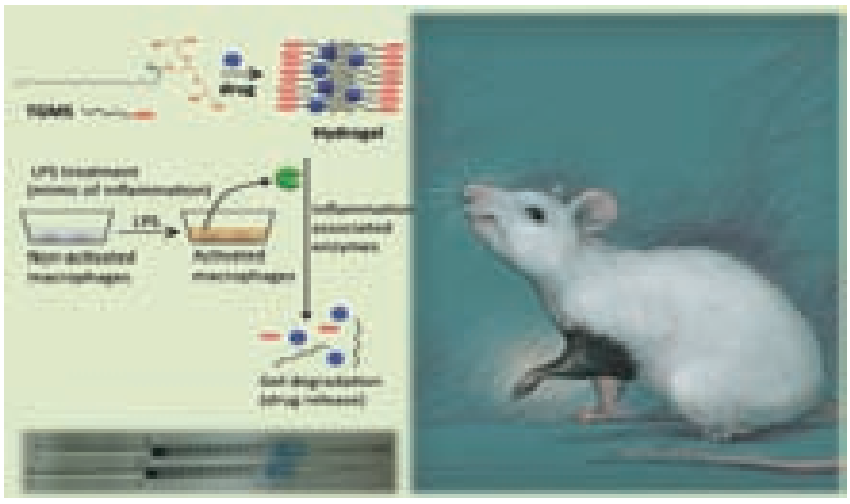


Fig.: Left panel: Schematic representation of hydrogel preparation and drug encapsulation through self-assembly process. Inflammation associated enzymes mediated drug release in an on-demand manner.

Right panel: Artist illustration of white-haired rat with transplanted black-haired rat limb.

RAMKUMAR SAMBASIVAN

Kouichi Hasegawa

The goal of regenerative medicine is to repair or replace damaged or diseased tissues or organs. Pluripotent stem cells, including embryonic stem (ES) cells (ESCs) and induced pluripotent stem (iPS) cells, retain the potency to differentiate into almost all cell types in embryos and adult body, and the ability for unlimited growth with normal genetics. The discovery of human pluripotent stem cells (hPSCs) has opened up the possibility for transplantation therapy and drug screening as well as disease mechanism studies.

Our research focus is on fundamental questions related to hPSC potency and technology development for hPSC engineering by integration of cell biology, developmental biology and material sciences. In an institutional collaboration between inStem and iCeMS, Kyoto University, Japan the group has established hPSC bulk culture systems by utilizing nano-fiber technology and functional polymers for control hPSC potency. More recently, the group also found several key intracellular signaling pathways involving in hPSC potency and growth, and identified chemical compounds can control these pathways.

Another major focus in the group is disease modeling with hPSCs to understand disease mechanisms and build a platform for drug screening. In collaboration with scientists in inStem, NCBS and other institutions, our group started establishing cardiomyopathy disease modeling

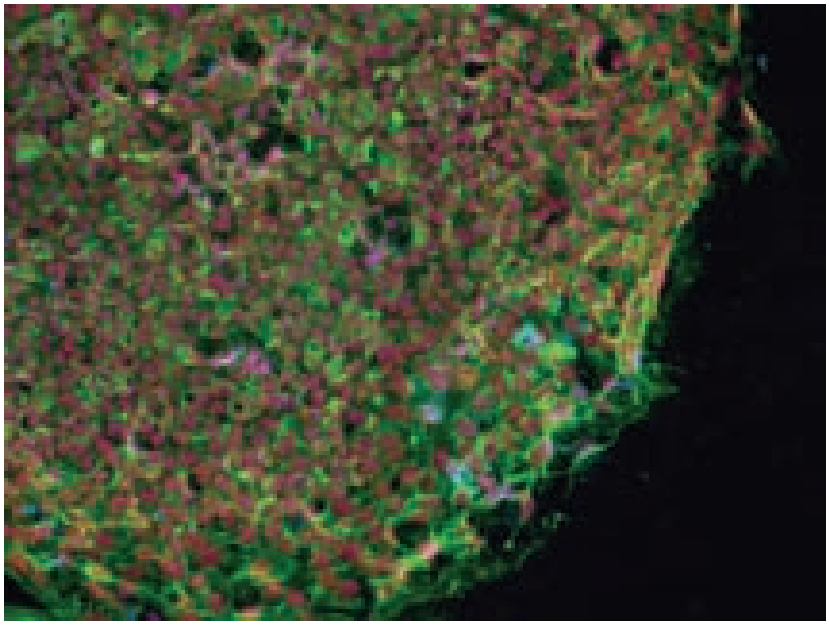


Fig.: Pluripotent marker expression in generated iPS cells. The IPS cell colony was stained with pluripotent transcription factor OCT4 (red) and cell surface marker TRA-1-81 (green).

and malaria infection modeling by generating hPSCs from patients and differentiating the hPSCs into cardiomyocytes and hepatocytes. In addition, our group generated embryonic pancreatic progenitor cells from hPSCs for exploring pancreatic regeneration by signaling pathway analysis in their growth and differentiation, as well as identifying biomarkers of pancreatic cancer by using similarity between the progenitors and cancer cells in pancreas. This has been reported in a book chapter about overview of regenerative medicine in pancreas including our data of pancreatic differentiation from hPSCs.

In addition, we established the inStem hPSC facility, a central hub of hPSC research. In 2013-14, researchers from 5 laboratories in 3 themes were registered and started hPSC research in the facility. We are providing quality-controlled hPSCs for the users to conduct stem cell and biomedical research at inStem. We standardized and started providing several key technologies for working with hPSC, such as iPSC generation, cardiomyocyte differentiation, hepatic and pancreatic differentiation. The facility will facilitate and accelerate research on stem cell and regenerative medicine at inStem.

Ramkumar Sambasivan

A key feature of vertebrate evolution is the acquisition of a head. This marked the shift from filter-feeding to active predation contributing to the success of vertebrates in colonizing the earth. Novel embryonic cell types unique to vertebrates generate most of the vertebrate head. Whereas the paired sensory organs are generated from placodes (thickenings in the ectoderm germlayer), the associated neural circuits, supportive skeleton and a variety of other tissues derive from cranial neural crest (CNC). Both these cell types are unique to vertebrates. Broadly, we aim to understand the mechanistic basis for the evolutionary origin of novel embryonic cell types.

CNC derives from ectoderm and generates ectodermal cell types neurons and glia. Remarkably, it also generates a variety of tissues including skeleton which are normally mesoderm derivatives in vertebrates. We are investigating the genetic programme underlying the mesodermal character of CNC. In a candidate approach, we are identifying the gene network established by Twist1, a developmental transcription factor (TF). Twist1 is a key mesoderm factor, but is also expressed in CNC. In a systematic approach, using small RNA sequencing, we are working to identify miRNAs that regulate the binary ectodermal versus ‘mesodermal’ choice of CNC. Together, our studies will reveal the basis of novel developmental potential of CNC and provide insight into its origin.

While placodes and CNC are accepted in the field to be vertebrate novelties, our research strongly indicates that cranial / head mesoderm is also an evolutionarily novel, vertebrate-specific embryonic tissue. Head mesoderm generates most of the head muscles. Using a mutant mouse strain, we find that loss of function of **Tbx6,** encoding a critical developmetal TF, known to affect the trunk mesoderm dramatically spares head mesoderm development (See Figure). This observation points to an independent developmental programme for head mesoderm compared to trunk supporting its vertebrate origin. We are corroborating this finding using multiple approaches including tracing the divergence in the cell lineage of head and trunk mesoderm. We will also perform comparative molecular studies in organisms that serve as proxies for basal vertebrates (lamprey) and vertebrate ancestor (Amphioxus) to trace the emergence of head mesoderm tissue in the vertebrate phylogenetic lineage. In addition, we are studying the signaling pathways that act upstream of **Tbx6** to specify head mesoderm distinct from trunk mesoderm. Furthermore, we are systematically investigating the differences in the head and trunk mesodermal gene regulatory network established by **Brachyury** and **Tbx6**. These studies will provide the strongest evidence for origin of head mesoderm independent of trunk mesoderm in the vertebrate lineage. In summary, our work will shed light on the fundamental mechanisms underlying the origin of novel cell types and on the evolution vertebrate head.

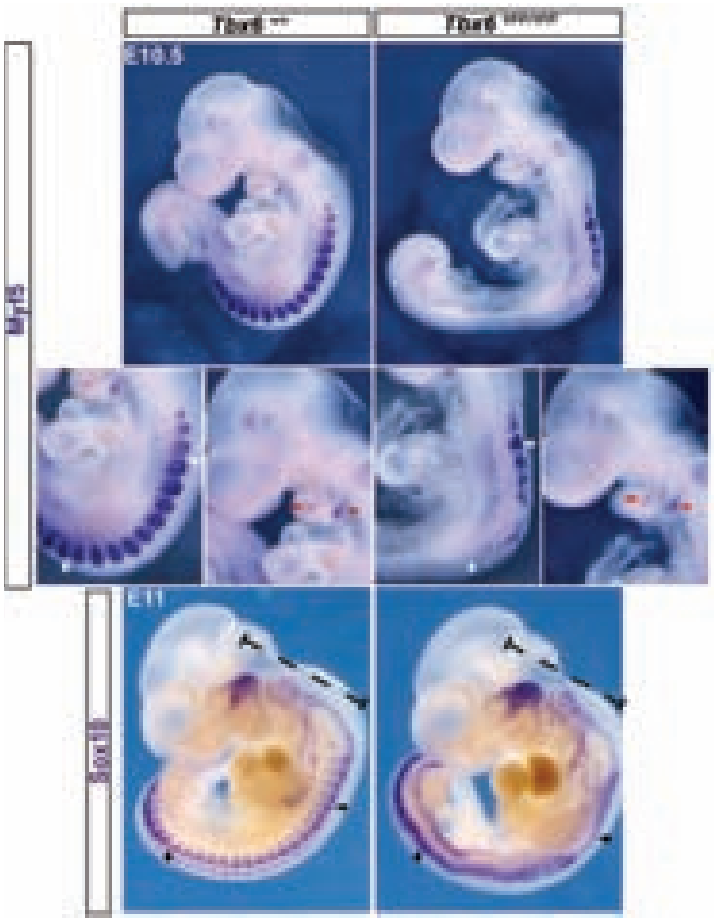


Fig.: Tbx6 mutation highlights divergence of head mesoderm development from that of trunk. Mouse embryos stained to detect of Myf5 and Sox10 transcripts by in situ mRNA hybridization. Myf5 is a marker of developing mesoderm. Sox10 is a neural crest marker, which indirectly reports for mesoderm development. E10.5 and E11 indicate the embryonic stage. Note dramatic failure of trunk mesoderm (white arrows for Myf5 or black arrows for Sox10 panels). In start contrast, head mesoderm develops normally (red arrows for Myf5 or dashed line for Sox10 panels).

Dasaradhi Palakodeti

RNA binding proteins are essential for cell fate decisions during planarian regeneration.

Planarians have emerged as an efficient model system to study regeneration and stem cell biology. They are capable of regenerating any missing tissues and organs lost due to amputation or injury. Their remarkable regenerative prowess comes from specialized cells, called Neoblasts, which are pluripotent in nature. Upon amputation or injury, Neoblasts proliferate rapidly and differentiate to form an undifferentiated, unpigmented tissue, called blastema, at the site of amputation. The blastema grows and differentiates further to form missing tissues and organs. My lab is interested in understanding post-transcriptional regulatory networks essential for stem cell function and regeneration in planaria.

In the last one year, we have identified several RNA binding proteins that are essential for regeneration and stem cell function. Highlights of our findings are mentioned below.

1. From transcriptome data, we identified several RRM (RNA Recognition Motif) and DEAD box helicase proteins that are enriched in the Neoblast populations.
2. SMED-PABPC2 and SMED-PABPN, RNA recognition motif (RRM) domain containing proteins are essential for epidermal integrity and muscle regeneration in planarians respectively.
3. SMED-PABPC2 is also essential for localized proliferation of Neoblasts during planarian regeneration.
4. DEAD box helicase, DDX24 is essential for neuronal and germ-line specification during planarian regeneration
5. DDX24 regulates genes expressed during the initial phase of wound healing, which might be essential for inflammatory response after amputation/injury.

Thus our studies provide insights into the roles of RNA binding proteins in stem cell function, cell fate decision & regeneration.

Akash Gulyani

Cell fate and physiology are regulated by highly complex and dynamic signaling networks. For instance, the activity of key signaling proteins is localized, both spatially and temporally in cells and tissues. Moreover, the same signaling proteins carry out multiple functions in cells, often leading to opposing outcomes. Understanding this complexity requires molecular sensors and tools that can provide quantitative information in intact, living cells and tissues. (For background and our new approach see Gulyani et al Nature Chemical Biology 2011)

In the last year, we have established a robust platform to generate sensors and modulators of signaling proteins, using high throughput screening of engineered protein libraries. Using yeast display technology, fluorescence activated cell sorting (FACS) and systematic high throughput screening; we have developed new, highly specific monobody (antibody mimics) binding proteins for several Src family kinases (SFKs). For instance, we have a monobody that binds only to Fyn but not to any of the other eight, highly homologous SFKs **(Fig.1)**. Src kinases control multiple aspects of cell fate, including stemness and differentiation, cell migration and proliferation but it's not clear how. Also, the role of individual Src family members is not clear. These newly developed binding proteins and sensors are currently being used to both measure and perturb Src kinase activity in living cells. This effort is a strong collaboration with Professor B.M. Rao of North Carolina State University, USA. We are working closely with other researchers at inStem and NCBS, including Drs. Ravi Muddashetty, Colin Jamora, Ranabir Das and Dasaradhi Palakodeti to build other novel sensors and tools for signaling dynamics in multiple cellular and disease contexts.

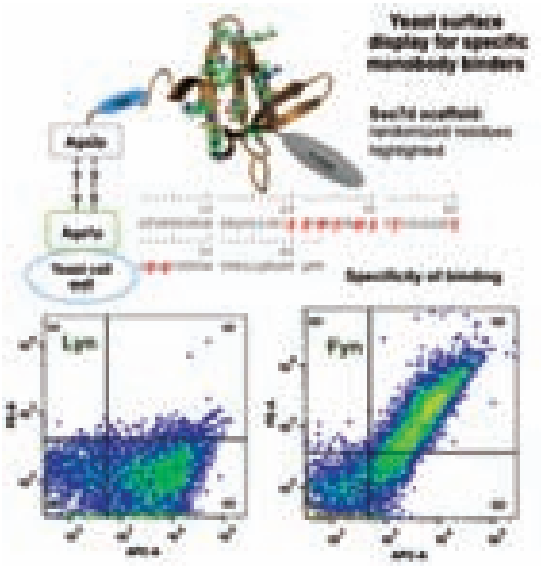


Fig.: High throughput screening of protein libraries for sensors and modulators. FACS data shown here provides an example of a monobody binder that binds only to Fyn kinase while showing no binding to closely related Src family kinases.

The model organism planaria shows a remarkable ability to regenerate after injury or amputation. For instance, after decapitation, planaria is able to regenerate its entire dorsal ganglion ('brain'), sensory organs (including eyes) and network connections with the intact ventral nerve cord within 7-8 days. Using precise optical stimulation

and quantitative measurements, we have recently uncovered dramatic new evidence of multiple kinds of light sensitivities in planaria, including ocular (eye based) and extra-ocular (all over the body) photosensitivity. With these measurements of light sensitivities, including the interplay between the ocular and extra-ocular sensitivities, we have been able to map the regeneration of the planarial neuronal networks in new ways. For instance, we have been able to breakdown the apparently linear process of regeneration into specific phases, using assays that have very simple binary readouts. These finding have allowed us to ask questions about neuronal regeneration, patterning as well as functional elements of a neural network.

Jeff Abramson

Membrane proteins comprise ~30% of the cellular proteome and perform many essential functions in the cell including signal transduction and the transport of diverse group of molecules. In spite of their biological importance, less than 1% of structures in the PDB are of membrane proteins. The reason for this discrepancy arise from their hydrophobic nature making them difficult to express, purify and structurally resolve. The Abramson lab at INSTEM has focused on three main areas to overcome these obstacles and determine the biological nature of transport from a number of membrane protein families.

I. DEVELOPMENT OF NOVEL PROTEIN EXPRESSION AND CRYSTALLIZATION METHODS

- Utilizing GFP-fusion based high throughput cloning and expression system in bacteria and yeast to monitor the level of protein expression and stability in diverse set of detergents.
- Developing lipid-based crystallization methods to improve the likelihood of obtaining well-diffracting crystals.
- In collaboration with Dr. BM Rao (NCSU), we have also initiated a project to identify conformation specific binders for co-crystallization with membrane proteins.

II. ELUCIDATING THE TRANSPORT CYCLE OF SUGAR TRANSPORTERS

- Implementing biophysical techniques—electron paramagnetic resonance (EPR) and solution x-ray scattering—in combination with crystallography and molecular dynamics to discover mechanistic details of sugar transport.

III. PURIFICATION AND CRYSTALLIZATION OF NUCLEOTIDE SUGAR TRANSPORTERS

- Nucleotide sugar transporters (NST) of the SLC35 family (solute linked carrier) are essential eukaryotic membrane proteins responsible for the transport of nucleotide sugars from the

cytosol to their ultimate destinations in the ER and Golgi apparatus. NST's are integral membrane proteins, which function as anti-porters of nucleotide sugars and their corresponding nucleotide mono-phosphates. Utilizing the techniques discussed in i., high throughput screening of NST orthologs is in progress to identify targets for crystallization.

In December 2013, we conducted a workshop titled "Practical aspects of membrane protein crystallography" which was attended by 24 students and postdocs from all over India. Attendees gained hands-on experience in advanced membrane protein crystallization techniques. We hope to conduct this workshop annually and use it as a forum for interaction between various membrane protein research labs in India.

Publications

1. Sahadevan, S., Antonopoulos, A., Haslam, S. M., Dell, A., Ramaswamy, S. and Babu, P. (2013) Unique, Polyfucosylated Glycan Receptor Interactions Are Essential for Regeneration of Hydrammagnipapillata, ACS Chemical Biology, 9,1,147-155.
2. Singh, D., Kumari, A., Ramaswamy, S. and Ramanathan, G. (2014) Expression, purification and substrate specificities of 3-nitrotoluene dioxygenase from Diaphorobacter sp. strain DS2, Biochemical and Biophysical Research Communications, 445,1,36-42.
3. Gallat, F.X., Matsugaki, N., Coussens, N.P., Yagi, K.J., Boudes, M., Higashi, T., Tsuji, D., Tatano, Y., Suzuki, M. and Mizohata, E. (2014) In vivo crystallography at X-ray free-electron lasers: the next generation of structural biology?, Philosophical Transactions of the Royal Society B: Biological Sciences, 369,1647,20130497.
4. Gangi, S.T., Cho, C., Govindappa, S., Apicella, M.A., Ramaswamy, S. (2014) Bacterial periplasmic sialic acid-binding proteins exhibit a conserved binding site, Biological Crystallography, 70,7.
5. Sadasivam, S. and DeCaprio, J. A. (2013) The DREAM complex: Master coordinator of cell cycle dependent gene expression. Nature Reviews Cancer 13, 585-595. (Inspired the cover page art: "Sweet Dreams" of the same issue)
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   (#Denotes equal contribution, *Corresponding authors)

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9. Liu, L., Yoshioka, M., Nakajima, M., Ogasawara, A., Liu, J., Hasegawa, K., Li, S., Zou, J., Nakatsuji, N., Kamei, K. and Chen, Y. (2014) Nanofibrous gelatin substrates for long-term expansion of human pluripotent stem cells. Biomaterials, 35(24) 6259-6267.
10. Sarkar, S.D., Yoshida, N. and Hasegawa, K. (2014) Overview of pancreatic replacement of β-cells from various cell sources. Stem Cell Therapy for Organ Failures, Editor S. Indumathi, Springer press, (in press).
11. Rangiah, K. and Palakodeti, D. (2013) Comprehensive analysis of neurotransmitters from regenerating planarian extract using an ultrahigh-performance liquid chromatography/ mass spectrometry/selected reaction monitoring method. Rapid Commun Mass Spectrom. 15;27(21):2439-52.
12. Sasidharan, V., Lu, Y.C., Bansal, D., Dasari, P., Poduval, D., Seshasayee, A., Resch, A.M., Graveley, B.R. and Palakodeti, D. (2013) Identification of neoblast- and regeneration-specific miRNAs in the planarian Schmidtea mediterranea. RNA. 19(10):1394-404.
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15. Abramson, J. and Vartanian, A.S. (2013) Biochemistry. Watch water flow. Science. 14;340(6138):1294-5. doi: 10.1126/science.1239270.

Honours and Awards

RAMASWAMY

1. Swagatha Ghosh, graduate student: Best poster award in BioQuest 2013 (International Conference on Biotechnology for innovative applications) conducted by Amrita Vishwa Vidyapeetham, Kerala.
2. Swagatha Ghosh, graduate student: Best poster award, Annual Talks, NCBS, January 2013.

HASEGAWA

1. Life Science Research Promotion 2014 “Novel pancreatic cancer biomarkers for diagnosis and target therapy” Takeda Science Foundation, Japan.
2. IKP-GCE (Phase 1) 2014 “Develop a sustainable Plasmodium vivax liver stage assay using human hepatocytes derived from induced pluripotent stem cells” Grand Challenges in Global Health, Bill & Melinda Gates Foundation, USA.
3. Asia-Oceania Collaborative Research 2014 “Understanding of biochemical, biophysical, and cellular mechanisms underlying inherited cardiomyopathies in South India” Kanae Foundation for the Promotion of Medical Science, Japan.
4. International Collaborative Research Researcher Exchange 2014 “Identification of biomarkers in pancreatic ductal carcinoma for diagnosis and target therapy” Daiichi Sankyo Foundation of Life Science, Japan.

Invited Talks

VEMULA

1. Daiichi Sankyo India Pharma Private Limited, Gurgaon, 11th July 2014. “A new paradigm in nanotherapeutics: Next-generation nanomaterials to prevent autoimmune diseases”.
2. Institute of Nano Science and Technology, Mohali, 10th July 2014. “Next-generation nanomaterials to prevent autoimmune diseases – A new paradigm in nanotherapeutics”.
3. 1st International Conference on Emerging Trends of Nanotechnology and Drug Discovery, Delhi, 26-27th May 2014. “Advanced nanomaterials to prevent autoimmune diseases: A new paradigm in nanotherapeutics”.
4. Centre for Stem Cell Research, CMC, Vellore, 23rd April 2014. “A new paradigm in nanotherapeutics: Next-generation nanomaterials to prevent autoimmune diseases”.
5. Faculty Entrepreneurship Programme by DST sponsored, Sri Venkateshwara University, Tirupati, 24th March 2014. “A tale of three (ad)ventures: My entrepreneurial journey”.
6. ICONSAT, International Conference on Nano Science and Technology-2014, Chandigarh, 2-5th March 2014. “Translational research through nanomedicine: Prevention of metal induced contact dermatitis”.
7. National Institute of Immunology-Max Planck Institute Workshop, at NII, New Delhi, 20-21st January 2014. “Translational research through nanomedicine: Prevention of metal induced contact dermatitis”.
8. Bangalore INDIA NANO 2013, 6th December 2013. “Role of nanotherapeutics in dermatology – prevention of metal induced skin allergies”.

9. Biotechnology Finishing School, Bangalore, 27th November 2013. "Translational research through nanobiomaterials".
10. India-France Technology Summit, New Delhi. 23rd October 2013. "Topical nanoparticles-based cream prevents metal induced skin allergies".

HASEGAWA

1. National Centre of Biological Sciences (NCBS) Annual Talks 2013. NCBS, Bangalore, India. January 3-5, 2013. "Control in human pluripotent stem cell self-renewal and differentiation".
2. Stem Cells Australia (SCA) – iCeMS Joint Symposium. The University of Melbourne. Melbourne, Australia. February 12-13, 2013. "Wnt signaling in human pluripotent stem cell self-renewal and differentiation".
3. International Symposium, Human Pluripotent Stem Cells: Progress to Therapy. The University of Sheffield. Sheffield UK. April 8-10, 2013 "Wnt signaling in human pluripotent stem cell self-renewal and differentiation".
4. University System Taiwan (UST) – iCeMS Joint Symposium, Kyoto University, Kyoto Japan, May 16-17, 2013. "Control of Human Pluripotent Stem Cell Self-Renewal and Differentiation".
5. University System Taiwan (UST) – Kyoto University International Symposium, National Chia Tung University, Hsinchu, Taiwan, November 17-19, 2013. "Technical Innovation of Human Pluripotent Stem Cell Biology Towards Regenerative Medicine".
6. World Stem Cell Summit 2014, San Antonio, USA, December 3-5, 2014. "Simple, defined and low cost culture medium for human pluripotent stem cell generation and expansion".

PALAKODETI

1. Indian Society of Developmental Biologists Conference, Mumbai, TIFR, December 1-4, 2013.
2. All India Cell Biology Conference, Bangalore, inStem, December 21-24, 2013.

GULYANI

1. University of Delhi, Central Drug Research Institute (CDRI), Lucknow, and Regional Centre for Biotechnology, Delhi.

Patents

RAMASWAMY

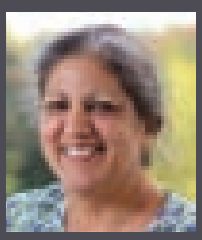
1. US patent (WiSys Ref: T100009US01) with subject "Blue Fluorescent Protein and Methods of Use".

8. PROGRAMME ON ADULT STEM CELL POTENCY

Quiescence and adult stem cell potency

In the adult mammal, most cells are non-dividing but exist in distinct arrested states. Whereas differentiated cells permanently withdraw from the cell cycle, rare resident stem cells idle in a dormant state known as quiescence. These temporarily arrested progenitors are responsible for both maintenance of adult tissue, coping with routine wear and tear as well as regeneration after injury. De-regulation of the quiescent state underlies both cancer (failure to enter arrest) and degenerative disease (failure to exit arrest), but is incompletely explored. 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 a cultured myoblast system that models muscle stem cells, we have uncovered active controls at multiple levels of gene regulation specific to quiescence and use this framework to investigate a variety of adult stem cells. Our studies indicate that quiescent cells preserve two antagonistic programmes (division vs. differentiation) in an inactive but primed state. Thus, rather than characterizing the quiescent cell as a "sleeping beauty" waiting to be awakened, a more appropriate metaphor is the "highwire artist", emphasizing the balancing act that these cells must execute to maintain the potency of cells for returning to either cell division or differentiation depending on the extrinsic milieu.

Over the past year, we have continued our investigations into the molecular control of adult stem cell quiescence using cultured cell lines that model quiescence, as well as primary mouse and human stem cells. As a means of deconstructing the quiescent state we have investigated the contribution of mechanisms at different levels. Some highlights of these studies are:



JYOTSNA DHAWAN

CHROMATIN AND TRANSCRIPTIONAL MECHANISMS IN Gθ

- A single epigenetic regulator PRDM2 regulates two key aspects of quiescence- repressed differentiation and poising of the cell cycle. In collaboration with Henrik Schroeder at Odense we find that Prdm2 is expressed in quiescent human muscle stem cells. We have confirmed that PRDM2 interacts with Rb as reported, and are now exploring the other interacting partners of PRDM2 using mass spectrometry.
- In collaboration with Rakesh Mishra at CCMB and Richard Harvey at VCCRI we are deciphering the rules that may govern the distinct chromatin landscape in quiescent cells. The results reinforce our view that global patterns of histone modifications induce a poised state in Gθ.
- In collaboration with Boudewijn Burgering at Utrecht, ChIP-seq evaluation of RNA polymerase stalling also point to quiescence-specific priming of the transcriptional programme.
- In collaboration with Ana Ferreiro at CNRS, we are studiying the links between chromatin state, redox stress, and muscle disease focusing on Selenoprotein N (SelN).

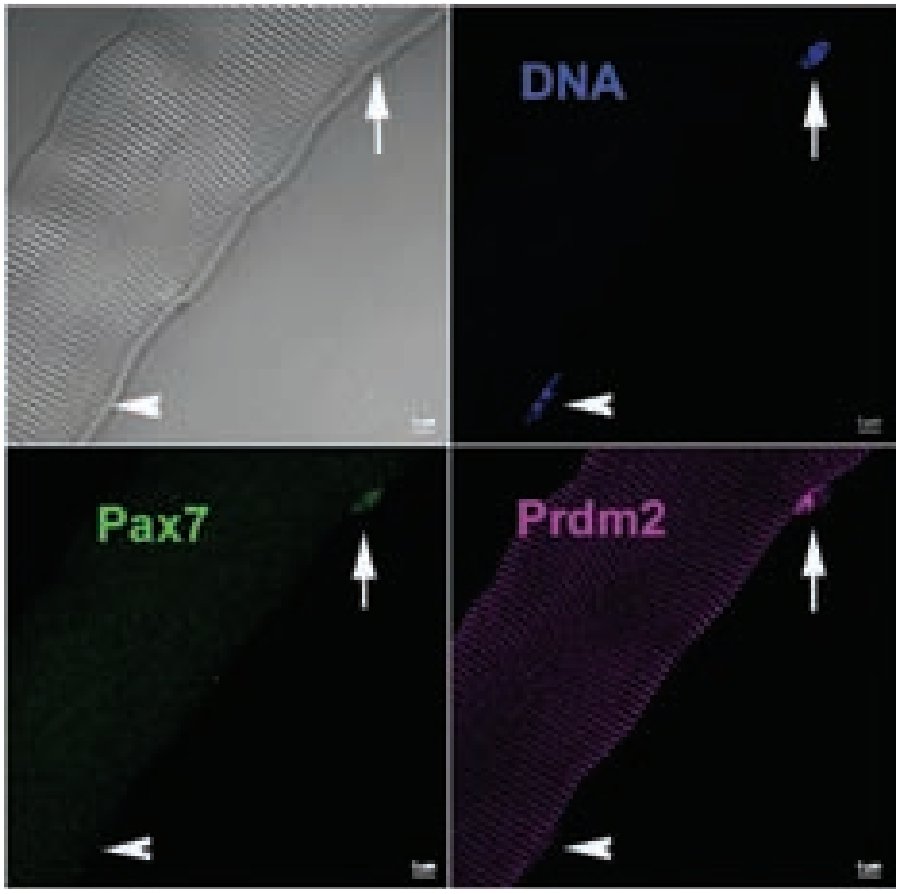


Fig.: Prdm2 – an epigenetic modulator identified in quiescent myoblasts in culture- is expressed specifically in quiescent muscle satellite stem cells in single muscle fibers isolated from adult mouse muscle.

Upper left panel-DIC image of a region of a single myofiber, note the striations typical of skeletal muscle; upper right-DAPI staining reveals two nuclei (arrow and arrowhead); Lower left –Pax7 immuno-staining idenfiies one nucleus as belonging to a satellite cell (arrow) while the myofiber nucleus is negative (arrow head); Prdm2 immuno-staining reveals that only the Pax7+ satellite cell expresses the chromatin modulator (arrow). Striated staining in myofiber is typical non-specific antibody background seen in this tissue.
Credit: Amena Saleh

RNA BIOLOGY IN QUIESCENCE

- We find distinct dynamics and composition of P-bodies in quiescent cells, indicating that transcript stabilization in mRNPs dominates over transcript turnover. Knock-down of different P-body components affects viability indicating a requirement for these processing bodies in quiescence.
- Gθ-specific miRNA profiling in collaboration with Das Palakodeti at inStem adds to the framework for how post-transcriptional mechanisms contribute to quiescence.

ENGINEERING QUIESCENCE BY MANIPULATING SIGNALS

- Culture systems that permit human mesenchymal stem cells to be toggled between quiescence and activation in vitro have not been previously characterized. In collaboration with Moustapha Kassem at Odense we have generated a new MSC quiescence model and uncovered transcriptional programmes associated with an altered secretory phenotype.
- Threshold levels of Wnt-β-catenin signaling are important for the quiescence programme and appear to be associated with a very different spectrum of genes in quiescent myoblasts when compared to either cycling or differentiated muscle cells.
- A primary cilium is extended in quiescent cells, and the centrosome is captured as its basal body. Our cilium knockdown studies support a quiescence-associated tumor suppressive role for this organelle.

Publications

1. Vishavkarma, R., Raghavan, S., Kuyyamudi, C., Majumder, A., Dhawan, J. and Pullarkat, P.A. (2014) Role of actin filaments in correlating nuclear shape and cell spreading. PLOS One (in press).
2. Srivastava, S., Sowpati, D.T., Garapati, H.S., Puri, D.R., Dhawan, J., Mishra, R.K. (2014) A ChIP-on-chip tiling array approach detects functional histone-free regions associated with boundaries at vertebrate HOX genes. Genomics Data 2 78-81.
3. Subramaniam, S., Prethish, S., Cheedipudi, S., Reddy, V.R., Shashidhara, L.S., Ravi Kumar, Ch., Mylavarapu, M., and Dhawan, J. (2013) Distinct transcriptional networks in quiescent myoblasts: a role for Wnt signaling in reversible vs. irreversible arrest. PLOS One 8(6):e65097.doi: 10.1371/journal.pone.0065097.
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5. Srivastava, S., Puri, D., Garapati, H.S., Dhawan, J. and Mishra, R. (2013) Vertebrate GAGA factor associated insulator elements demarcate homeotic genes in the HOX clusters. Epigenetics & Chromatin 6(1):8. doi: 10.1186/1756-8935-6-8.

Honours and Awards

1. Dr. Jyotsna Dhawan received a VASVIK award for Life Sciences (Smt. Chandaben Mohanbhai Patel Industrial Research Award for Women Scientists) Dec 2013.

9. CENTRE FOR STEM CELL RESEARCH

The Centre for Stem Cell Research (CSCR) is a unit of inStem, Bengaluru at the campus of the Christian Medical College, Bagayam, Vellore. CSCR has its focus on translational research with stem cells aimed a developing novel therapies for human diseases or understanding disease biology using novel models. Scientists at CSCR either have clinical attachment themselves or work in close collaboration with medical colleagues in CMC, Vellore to keep their within the above mandate. The summary below provides a snapshot of the major programmes and research projects being pursued at CSCR. The two most advanced from the translational therapeutic perspective are the gene therapy and musculoskeletal regeneration programmes. Further details of the programmes are available in the full annual report of CSCR and on its website (www.cscr.in).

1. **GENE THERAPY PROGRAMME: THIS GROUP CONSISTS OF DR. G R JAYANDHARAN (UP TO SEPTEMBER, 2014), DR. RV SHAJI AND DR. ALOK SRIVASTAVA FROM CSCR AND CMC, VELLORE WITH SEVERAL EXTERNAL COLLABORATORS. THIS PROGRAMME CURRENTLY CONSISTS OF TWO COMPONENTS**

   a. **Adeno-associated virus (AAV) vectors**

      Vector Biology and Pre-clinical studies: In this area, the laboratory led by Dr. G. Jayandharan, has developed various novel AAV based delivery systems through an understanding of biology of virus and host cellular interactions. An array of nearly 60 novel capsid variants based on AAV serotypes 1-10 has been generated by strategic modification of capsid ubiquitination sites. These capsid modified forms with enhanced efficiency and reduced immunogenicity have the ability to target multiple tissues and thus applicable for different diseases. Intellectual property rights (IPR) have been filed on these vector and need to be followed up. Currently, it appears though that one of the wild type AAV 8 or 3 may be the best vector, free of IPR restrictions, for hemophilia to take forward to the clinic for several reasons.

      Gene Therapy for Hemophilia: Other Collaborators: Dr. Alok Srivastava has established collaboration with Dr. Arun Srivastava, University of Florida, USA and Dr. Amit Nathwani,

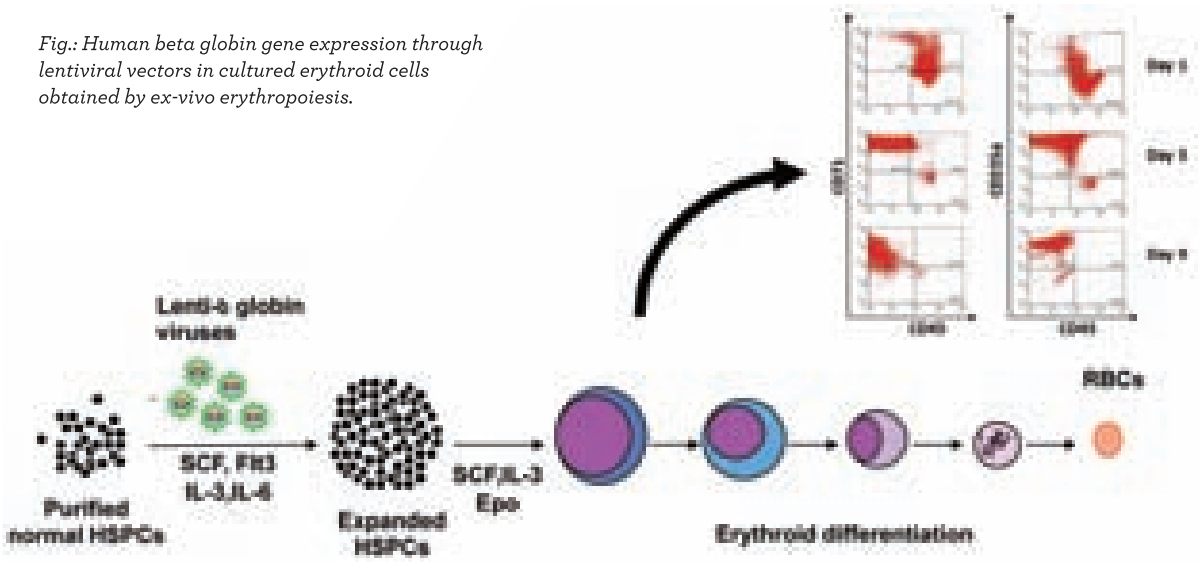
University College London, UK. Dr. Arun Srivastava has recently shown that AAV3 WT has much greater tropism and efficiency for transducing human hepatocytes compared to AAV8 and is keen to collaborate with us for its clinical development. Dr. Amit Nathwani continues to be one of the lead investigators in the world for clinical trials of gene therapy for hemophilia and is also keen to include CSCR/CMC, Vellore in those trials. One of the major limitations in the field has been a good cost efficient scaled up production facility for vectors. We have also established collaboration with Dr.Urmish Chudgar of INTAS Pharmaceuticals, Ahmedabad for this purpose. As the clinical programme evolves, the team from CMC, Vellore involved in the management of patients with hemophilia will become part of this programme.

Dr. Alok Srivastava is also working with the ICMR and DBT for evolving the regulatory processes for the review, approval and monitoring of any gene therapy clinical trial in India.

b. Lenti viral vectors for thalassemia gene therapy

A collaboration has been established between Dr. Alok Srivastava and Dr. R V Shaji at CSCR with Dr. Trent Spencer at Emory University, USA for developing lenti viral vector based gene therapy for thalassemia. At this time, preclinical studies are being carried out by Dr. R V Shaji in an ex-vivo erythropoiesis system with different vectors designed by the group.

Fig.: Human beta globin gene expression through lentiviral vectors in cultured erythroid cells obtained by ex-vivo erythropoiesis.



2. MUSCULOSKELETAL REGENERATION PROGRAMME – THIS GROUP IS LED BY DR. VRISHA MADHURI AT CSCR AND CMC, VELLORE WITH SEVERAL COLLABORATORS IN CSCR AND CMC ALONG WITH MANY EXTERNAL COLLABORATORS.

This group's focus is on musculoskeletal regeneration with the major targets being articular and physeal cartilage replacement, bone and muscle regeneration in different clinical conditions with otherwise limited therapeutic options. Over the last year, this group has completed, a large animal study has been completed on physeal replacement using chondrocytes with chitosan hyaluronic acid aldehyde (CHDA) gel. Based on this data, a clinical is being planned. Ongoing studies include long term follow-up children treated for physeal defects as well as a new large animal model for evaluation of a novel combination of polycaprolactone and CHDA scaffold for treating articular cartilage defect on the femoral head. A long planned phase 1 trial on he treatment of large bone defects using hydroxyapatite scaffold loaded with MSC differentiated to osteogenic lineage after obtaining final regulatory approvals. Another area of evolving work is with human muscle satellite cell isolation, culture and characterisation. We have developed the GMP protocols for the same and have also planned for studies on sphincter muscle regeneration (urinary continence, fecal incontinence).

3. BASIC RESEARCH PROGRAMMES

a. **Stem cell niche and cell fate programme**

This is an evolving area of work at CSCR with a large number of investigators involved. This area is the major focus of work of Dr. Aparna Venkatraman who studies this in two models – the gastrointestinal system (evaluated in the mouse model of inflammatory bowel disease as well as human intestinal biopsies) as well as the hematopoietic system in human bone marrow samples. Early data shows that though total colonic epithelial cell show a significant reduction in the number of immature epithelial cells in the lower crypt with a concomitant increase in the mature upper crypt cells in an animal model of colitis. Along with this decrease in number of immature crypt cells, aberrant cell migration, block of cell differentiation and cell cycle arrest were also noted. In parallel, an alteration of phenotypic number and migration in the surrounding mesenchyme was seen.

In elucidating the role of hematopoietic stem cells (HSCs) and their associated niche in development of MDS, Dr. Venkatraman has been able to show a block in differentiation among different grades of MDS patients. In parallel, in situ analysis of bone marrow trephine of low risk MDS samples for different niche components revealed altered vasculature and

clustering of HSPCs around vessels. At the molecular level, an increased expression of membrane bound β-catenin in vessels interacting with cadherin expressing HSPCs was noted. These data reveal that aberrant Wnt signaling in the vessels and HSPCs could be involved in the etio-pathogenesis of MDS.

Dr. Sanjay Kumar is also involved with this area of work and studies the stromal elements of the niche in the hematopoietic system along with Dr. Alok Srivastava who coordinates this work bringing in several collaborators from CMC, Vellore.

b. Regulation of hemoglobin synthesis and erythroid differentiation

Dr. R V Shaji uses an ex-vivo erythropoiesis model to study the role of cis-acting genetic elements in the beta globin cluster in patients with sickle cell disease with low and high foetal hemoglobin. We are currently carrying out experiments to understand the transcriptional regulatory roles of the regions that contain these sequence variations using DNAse hypersensitivity assays using cultured erythroid cells obtained by ex-vivo erythropoiesis. In addition, he also uses third generation inducible lentiviral vector and the new algorithms of snRNA design to generate a library of validated shRNAs aimed identification of epigenetic factors involved in somatic cell reprogramming and human erythropoiesis.

c. iPS technology & disease modeling

Dr. R V Shaji also uses induced pluripotent stem cell technology to model diseases using a variety of gene transfer methods: retroviral, lentiviral and episomal and by using Sendai virus. We are currently generating hiPSCs from patients with haematological disorders. Using doxycycline inducible lentiviral vectors to express Fanconi anaemia genes we generated iPSCs from patients with Fanconi anaemia. These iPSCs will be differentiated to different lineages to understand the role of Fanconi anaemia pathway in different human cell types.

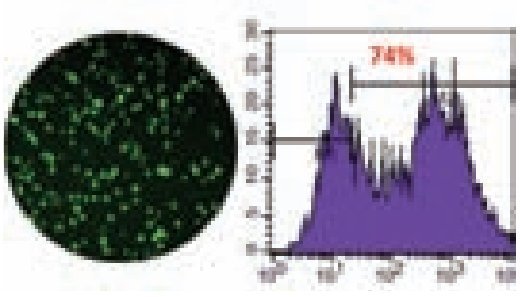


Fig.: Lentiviral expression of GFP in CD34+ cells

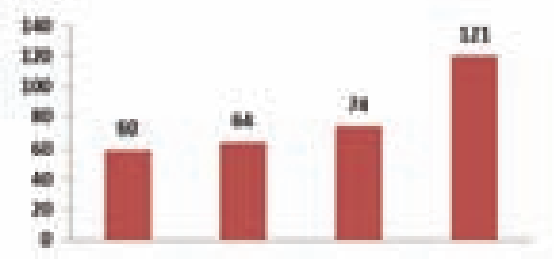
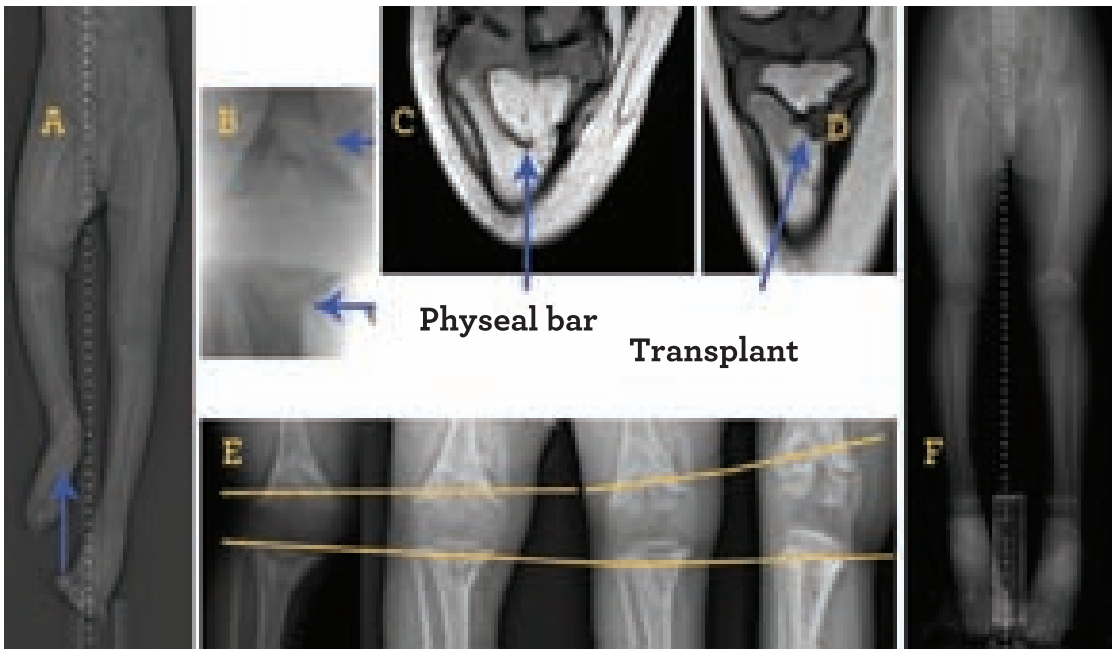


Fig.: Lentiviral expression of human b globin gene in different MOIs.

d. **Vascular biology programme**

Dr. Rekha Samuel focuses on dissecting pathophysiological/molecular mechanisms of microvascular dysfunction in Type-2 Diabetes (T2D) using the gestational diabetes placenta as a model. She also generates adipose tissue derived stable functional vasculature and evaluates vasculopathy in scleroderma. Functionality of human engineered blood vessels is examined in severe combined immune deficient (SCID) mice. These studies target understanding inherent endothelial dysfunction in vascular disease as well as the role of other elements such as pericytes.



Preoperative

2 year post-operative

Fig. A-F Autologous culture expanded chondrocytes transplant for growth arrest following neonatal sepsis in a 2.5 year old.
A. CT Scanogram shows arrest of Right lower femur and both upper tibia physis with severe shortening of right lower limb.
B. Shows intraoperative radiographs of surgical opening in the damaged area of physes for transplant.
C & D. Show the growth of the upper tibia physis before and after transplant on MRI.
E. Shows increasing growth at the damaged physis following surgery for 2 years.
F. Final scanogram at 2 years show restoration of limb length of the right lower limb and length equalisation.

e. **Mesenchymal stromal cells**

Dr. Sanjay Kumar studies the biology of mesenchymal stromal cells and their potential therapeutic applications. These include genetically-engineered human umbilical cord-derived mesenchymal stem cells (UC-MSC) exosomes as therapeutic delivery vehicles for tumor-targeted therapy or maintaining tissue-homeostasis and biological studies on chromatin modulators for MSC osteogenic fate choices in metabolic bone diseases. He also studies disease models such as spinal cord injury in SCID mice model using hPD-MSC/ neuro-progenitors and/or PTEN modulation in axons by inducible shRNA and therapeutic applications of genetically manipulated human term-placenta-derived mesenchymal stem Cells (PD-MSCs) as drug cells for treating acute radiation sickness (ARS) and/or radiation-induced cutaneous damages.

f. **Biomaterials and tissue engineering**

Dr. Murugan Ramalingam focuses on synthesis, design and characterization of biomedical materials and scaffolds suitable for controlling stem cell fate and function, and for engineering tissues and organs. We also investigate the basic cellular functions with respect to 2-D and 3-D microenvironments and then engineering tissues and organs in vitro.

Publications

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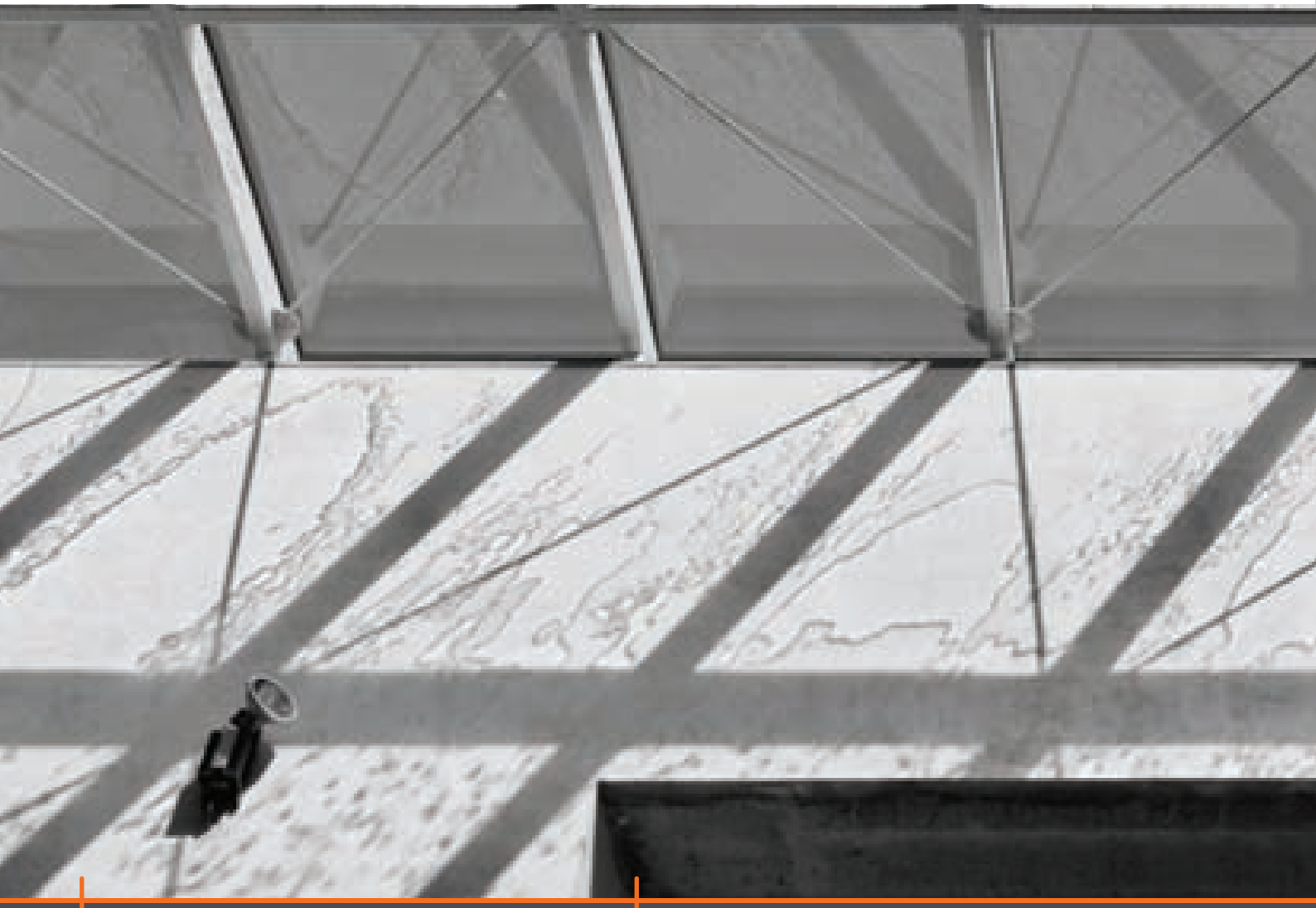
Patents

1. Novel AAV vectors- IN 1714/CHE/2012, US 13/886,241, European Union EP13166332.0
   INVENTORS: Jayandharan GR, Dwaipayan Sen, Sangeetha Hareendran, Nishanth Gabriel, Ruchita Selot, Akshaya K, Balaji B, Alok Srivastava [CMC, Vellore], Sudha Govindarajan, Rupali G, N Srinivasan [IISc, Bengaluru]
2. NF-kB in joint disease- India E-2/10024-2013-CHE
   INVENTORS: Jayandharan GR, Dwaipayan Sen, Aaron Chapla, Viju Daniel, Alok Srivastava, Noel Walter [CMC, Vellore].
3. Indian complete Patent Application No. 57/CHE/2014. Dated 6th January 2014.
   A process of labelling cells and a method of tracking thereof.
   INVENTOR: Sanjay Kumar.



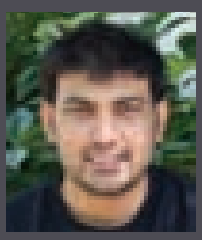
10. NEW INVESTIGATORS

Minhaj Sirajuddin

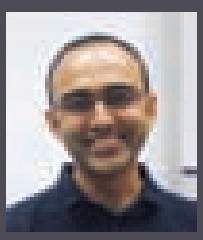
STRUCTURE AND FUNCTION OF CYTOSKELETAL ASSEMBLIES

Cytoskeletal assemblies such as actin and microtubules provide the framework for maintaining cell integrity and powers biological motions. Few examples of biological motions include; heart and muscle contraction, sperm motility and intracellular transport of cargos in a meter long neurons. Due to their important functions in eukaryotic cell physiology, mutations in cytoskeletal components have been linked to a variety of human diseases, such as cardiomyopathies, muscular dystrophies and neurodegenerative diseases. My research interests include understanding the function of cytoskeletal assemblies using structural, biochemical and biophysical approaches.

As a part of cardiomyopathy team, my research will focus on bridging the knowledge gap between clinical findings and molecular mechanism underlying cardiomyopathy disease causing mutations. Most of these mutations are located in proteins that are highly enriched in the heart and are important sarcomere components, the basic unit of the cardiomyocyte responsible for generating contractile forces during the heartbeat. However, understanding how single point mutations contribute to the progression of cardiomyopathies remains a challenge. This is largely due to a lack of molecular understanding of how sarcomere proteins coordinate during muscle contraction. In order to address this, our lab will use purified recombinant sarcomere components, which will enable us to measure the collective biophysical properties of core sarcomere proteins and compare with disease mutations. Furthermore, the structural work will involve determining high-resolution structures of uncharacterized sarcomere proteins. These approaches will enable us to tackle longstanding conundrums in the muscle field and motor biophysics, and has the potential towards developing therapeutic intervention for cardiomyopathy diseases. My other research interests include understanding the function of microtubule and associated proteins. This work will majorly benefit from the recombinant tubulin expression developed during my postdoctoral work. Using this method, our lab will purify microtubules engineered with a particular post-translational modification and study the effects of microtubule track modification on intracellular cargo transport.



MINHAJ SIRAJUDDIN



SUNIL LAXMAN

Publications

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Sunil Laxman

NUTRIENT SENSING AND METABOLIC REGULATION OF CELL FATES

Depending on the specific context, a cell can undergo different fates ranging from division, differentiation and autophagy to a commitment to cell death. It is now emerging that the metabolic state of a cell itself controls fundamental cellular processes, and thereby regulates cell fates. The metabolic state of a cell is controlled by available nutrients and the cellular "sensor" proteins or nucleic acids. These metabolic sensors respond to small changes in specific metabolites, and regulate global metabolic responses. There is an underlying hierarchy of nutrients (in the form of different carbon, nitrogen, sulfur and phosphorus sources) to which cells respond entirely differently, through different sensing systems.

Therefore, in order to understand how metabolism controls cell fate decisions, it is essential to identify the sentinel metabolites that control cell fates, and the nutrient/metabolic sensors that detect these metabolites. My research seeks to identify such sentinel metabolites and metabolic sensors, and the mechanisms through which they regulate cell fates. I am particularly interested in how cells sense and respond to amino acids. To address these fundamental questions, I combine the use of tractable genetic models, particularly but not limited to Saccharomyces cerevisiae, reconstitutive biochemical approaches, and quantitative metabolomic approaches. Some of these metabolic sensors have the potential to be targeted pharmacologically to switch cell fates. For example, a specific metabolite, through its sensor, could commit a cell towards quiescence. Modulating this sensor could however restore the ability of this cell to divide. Thus, these discovery based approaches are essential for our understanding of how cell fates are determined.

Publications

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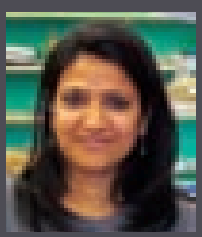

TINA MUKHERJEE

3. Laxman, S., Sutter, B.M., Wu, X., Kumar, S., Xiaofeng Guo, David, C., Trudgian, Hamid Mirzaei and Tu, B.P. (2013) Sulfur amino acids regulate cellular translational capacity through modulation of tRNA wobble-uridine thiolation. Cell, Volume 154, Issue 2, 416-429, 18 July 2013.
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Tina Mukherjee

METABOLIC AND SYSTEMIC CONTROL OF STEM CELL DEVELOPMENT

Recent studies have highlighted that multiple systemic stimuli, in addition to the well-studied cellular programmes, are important for stem and progenitor cell development. These stimuli include, signaling and metabolic inputs such as ROS, mitochondrial activity, hypoxia, amino acids, sensory inputs, and signals released from the brain such as neuro transmitters. My own findings, as well as results from other labs have uncovered the plausible mechanistic details and physiological relevance of some of these inputs in stem/progenitor cell maintenance and development.

THE GOALS OF MY RESEARCH INCLUDE

1. **Determining the role of metabolic control in Stem Cell Maintenance**
   Using ES cells and Drosophila stem cells we will investigate the role of metabolic pathways dictating cell fate decisions.

2. **Calcium homeostatic mechanisms in Stem Cells**
   Multiple stem cell types show sensitivity to extracellular environment such as nutrition, hypoxia, injury and others, which can directly translate to changes in their cell cycle profile and cell fate. We will test if homeostatic levels of Ca2+ in stem cells functions as a key sensor and mediator of perturbations in extracellular environment.

3. **Stress Signaling in Stem and Progenitor Cells**
   Current literature on mammalian immunity indicates that infections are less well tolerated under stress conditions. However, the signaling pathways activated upon different stress conditions and the relevance of the stem cell response to the development and survival of the organism are poorly understood. We will genetically define non-autonomous stress responses in stem cells.

Publications

1. Shim, J.*, Mukherjee, T.*,Mondal, B.C., Lui, T., Young, G., Wijeyvarnasuriya, D.P., Banerjee, U. (2013) Olfactory control of blood progenitor maintenance. Cell 2013, 155 1141-1153. *These authors contributed equally to the work.
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11. SHANTA WADHWANI CENTRE FOR CARDIAC AND NEURAL RESEARCH

The Shanta Wadhwani Centre for Cardiac and Neural Research (SWCCNR) at inStem was established in 2012, with generous support from the Wadhwani Foundation. The Center for Brain Development and Repair (CBDR) and the Centre for Cardiovascular Biology and Disease (CCBD) currently receive support from the Wadhwani Foundation as part of SWCCNR. The grant awarded via the Research and Innovation (RIN) division of the Wadhwani Foundation, has enabled both core research of the programme at inStem and also collaborative activities with exceptional scientists across the globe. This journey has been highly rewarding and has given substantial academic freedom to the researchers on the programme.

At CBDR, a team of researchers led by Sumantra Chattarji (NCBS), Sidharthan Chandran and Peter Kind (University of Edinburgh) majorly focuses on understanding autism spectrum disorders (ASDs) and intellectual disability (ASD/ID) at cellular and molecular levels in model organisms and in human-derived models. Neurobiologists at the Center have recently successfully generated and characterized human stem cell derived neurons and have performed electrophysiological recordings from live brain cells, which has paved the way for further discovery research. The Centre has also initiated analysis of newly generated transgenic rat models of Fragile X Syndrome.
The Cardiac Biology and Cardiomyopathy programme headed by Jim Spudich (Stanford) in collaboration with John Mercer (inStem) in collaboration with Norio Nakatsuji (iCeMS, Kyoto), Kouichi Hasegawa (iCeMS and inStem), R. Sowdhamini (NCBS) and Maneesha Inamdar (JNCASR and inStem) aims to study diseases related to sarcomeric proteins (actin, myosin, troponins, tropomyosin), the building blocks of the contractile unit present in cardiac muscle. The team has successfully developed the basic biochemical and biophysical assays to understand the activity of sarcomeric proteins like tropomyosin. To gain a comprehensive understanding of mechanism

of cardiac disease formation, Transcription activator-like effector nucleases (TALENs) are used to insert the disease-causing mutations into the genomes of embryonic stem cells (ESC) and in a new strain of humanised mice. This effort is essential for identifying therapeutic small molecules that will reverse the primary disease mechanisms. (http://wadhwani-foundation.org/)

12. ACADEMIC PROGRAMMES

Academic programmes and initiatives at the Institute for Stem Cell Science and Regenerative Medicine are shared with the National Centre for Biological Sciences and rooted in interdisciplinary research, fostering collaborations that span multiple disciplines. The graduate programme leading to the award of a PhD degree, opportunities for talented post-doctoral researchers and internships for undergraduate students are key features of the programme.

Selections for the PhD programme at inStem are through the entrance exam (JGEEBILS) and interviews conducted jointly with NCBS at the Bangalore Campus. The graduate programme offers students' flexibility to explore questions in research areas of their choice in our laboratories and in collaboration with our international partners. We are delighted that the past year saw inStem admit the first batch of students to its PhD programme. Recognizing the need for a vibrant Postdoctoral Programme for the success of its collaborative research themes, inStem has successfully sponsored post-doctoral fellows for competitive early career awards from the Wellcome Trust-DBT Alliance, DBT and DST and offers intramural funding opportunities for early career researchers.

At the core of the academic curriculum lies the in-house coursework programme, which is complemented by a range of seminars, meetings and workshops conducted year-round on campus. Our graduate students design individual programmes of study from courses offered in-house as well as in neighbouring Institutes in Bangalore. Our academic programmes are ably supported by Library Facilities subscribing to a large number of journals covering diverse fields of research and bibliography search support, with Internet access on campus and residential space and e-mail also provided. Participation in international meetings and workshops both within the country and outside is actively encouraged.

The heavily subscribed internship programme on our campus, offers undergraduate students within the country and from (though our international partners) outside, research and training opportunities for the short or longer-term in our laboratories.

Apurva Sarin
Head, Academic Activities

13. INSTEM FACULTY

Core Faculty

S. Ramaswamy	Senior Professor
Jyotsna Dhawan	Senior Professor
Srikala Raghavan	Assistant Investigator
Shravanti Rampalli Deshpande	Assistant Investigator
Akash Gulyani	Assistant Investigator
Colin Jamora	Associate Investigator[1]
John Mercer	Investigator
Kouichi Hasegawa	Assistant Investigator[2]
Kenichi Suzuki	Associate Investigator[2]
Dasaradhi Palakodeti	Senior Fellow
Archana Purushotham	Senior Fellow
Praveen Kumar Vemula	Senior Fellow
Ravi S. Muddashetty	Senior Fellow
Ramkumar Sambasivan	Senior Fellow

Visiting Faculty

Jeff Abramson (UCLA)	Collaborative Science Chair
Boudewijn Burgering (Utrecht)	Collaborative Science Chair
James Spudich (Stanford)	Collaborative Science Chair
Ashok Venkitaraman (Cambridge)	Collaborative Science Chair
Siddharthan Chandran (Edinburgh)	Collaborative Science Chair
Peter Kind (Edinburgh)	Collaborative Science Chair
Norio Nakatsuji (iCeMs)	Visiting Professor
Ramanuj Dasgupta (NYU)	Visiting Associate Investigator
Maneesha Inamdar (JNCASR)	Adjunct Faculty
Richard Morris (Edinburgh)	Visiting Professor
Anil Prabhakar (IIT, Madras)	inStem Associate

1. In collaboration with IFOM (Milan, Italy)
2. In collaboration with iCeMs (Kyoto, Japan)

14. INSTEM LEADERSHIP COMMITTEES

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